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***Vibrio parahaemolyticus* and the Seafood Industry**

R. NATARAJAN, MARTIN ABRAHAM and G. BALAKRISH NAIR

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608 502

The role of *Vibrio parahaemolyticus* in food borne gastroenteritis outbreaks associated primarily with the consumption of contaminated seafoods has been well documented. Information pertaining to various aspects of its occurrence in seafoods, procedures for isolation and identification, generation time and inactivation profiles is discussed. Emphasis has been given to the response of *V. parahaemolyticus* to low temperatures, heating and antibacterial agents. The public health hazard posed by the pathogen is outlined and the guidelines for control are reviewed in detail.

Advent of the "blue revolution" to combat protein malnutrition underlines an urgent need to probe into and ascertain the hazards of diseases transmitted primarily through edible marine species. Consequently quality control and microbiological safety of seafoods has of late, attained paramount importance. Partly because of the introduction of feeding programme on an extensive scale, increased consumption of precooked foods, importation of new sorts of feeds and partly because of improved laboratory techniques, outbreaks of food poisoning have burgeoned from a series of sporadic occurrences to epidemic levels (Wilson, 1973).

Vibrio parahaemolyticus has been incriminated since the 1950's, as the vehicle of numerous outbreaks of food borne gastroenteritis in Japan. Subsequent isolation of this halophilic pathogen in many maritime areas outside Japan triggered attempts to tackle the *V. parahaemolyticus* problem on an international basis. Although several research papers have been published on *V. parahaemolyticus*, information pertinent to the actual control of this organism is rather scarce (Lee, 1973). This paper highlights some idiosyncrasies peculiar to *V. parahaemolyticus* (whose rating as a human health hazard has undergone transformation from a mere "potential" to a grave "real") thereby outlining safety measures for seafood processors in particular and the public in general.

Occurrence

Being a mild halophile, the distribution of *V. parahaemolyticus* is chiefly confined to estuarine, brackish and coastal environs. Numerous workers have documented the occurrence of *V. parahaemolyticus* in various species of fin fish, shrimps, prawns, oysters, mussels, clams, crabs, snails and periwinkles (Krantz *et al.*, 1969; Leistner *et al.* 1971; Thompson & Trenholm, 1971; Kampelmacher *et al.*, 1972 Chatterjee & Neogy, 1972; Vanderzant *et al.*, 1973; Sutton, 1974; Chun *et al.*, 1974; Sizemore *et al.*, 1975; De *et al.*, 1977; Natarajan *et al.*, 1979 a, b). Suitable growth substrates for *V. parahaemolyticus* are however not limited to fish and shellfish alone as it can also grow on salted extracts of vegetables like cabbage, radish, lettuce, cucumber, potato and pumpkin. Kudoh *et al.*, (1974) in their paper on the epidemiology of food poisoning due to *V. parahaemolyticus* in Tokyo from 1963 to 1972, categorised the implicated food items as raw marine fish and shellfish (38.9%), foods containing fish and shellfish (38.9%), unknown (12%), processed sea products (6.4%) and vegetables and their products (3.6%).

Mariculture

Despite the fact that *Vibrio* spp. have long since been implicated in diseases and mass mortalities in cultured fish and shellfish, *V. parahaemolyticus* was not recognised until recently. Kusuda (1968)

suspected *V. parahaemolyticus*, alongside *V. alginolyticus* and *V. anguillarum*, to be the causative agents of an ulcer-like disease among cultivated fish in Wasaka Bay, Japan. Krantz *et al.*, (1969) observed that the haemolymph of moribund blue crabs, *Callinectes sapidus* consistently harboured higher counts of *V. parahaemolyticus* than their healthy counterparts. Vanderzant and Nickelson (1973) demonstrated that, under certain cultural conditions, *V. parahaemolyticus* caused death of laboratory reared postlarvae and adults of the brown shrimp (*Penaeus azetecus*). Eventhough *V. parahaemolyticus* poses a potential threat to maricultural operations, it must be emphasized that its direct pathogenicity to fish and shellfish is yet to be fully established.

Isolation and identification

Among the numerous isolation and identification schemes formulated for *V. parahaemolyticus* the most commonly employed one is that described in the Bacteriological Analytical Manual for Foods

Table 1a. *Analytical method for *V. parahaemolyticus*

Isolation procedure

Phase	Seafoods
	(Fish, shellfish and crustaceans) 50g + 450 ml 3% NaCl diluent (10° to 10 ⁻⁴ dilutions)
Day 1 (Enrichment)	Glucose salt teepol broth (Enumeration by 3- tubes most probable number method)
Day 2 (Isolation)	Thiosulfate citrate bile salts sucrose agar (Bluish-green colonies with darkened centres)
Day 3 (Screening)	Triple sugar iron agar Motility agar Trypticase soy agar Trypticase soy broth
Day 4 (Identification)	Biochemistry Serology

(Food Drug Administration Publication Washington, USA). The method which was evolved after an exhaustive study of the productivity of several Japanese and American techniques is briefly outlined in Tables 1a and 1b.

Table 1b. *Identifying characteristics

Test	Response
Gram stain	—
Morphology	Curved/straight rods
Motility	+
Triple sugar iron agar	K/A. H ₂ S (—), gas (—)
Hugh-Leifson glucose	Fermentation (+), gas (—)
Cytochrome oxidase	+
Arginine dihydrolase	—
Lysine decarboxylase	+
Gelatin	+
Halophilism (NaCl)	
6% & 8%	+
0% & 10%	—
Growth at 42° C	+
Voges proskauer	—
Indole	+
Cellobiose	—
Sucrose	—
Maltose	+
Mannitol	+
Trehalose	+

* Adapted from FDA By-Lines 4 (2), Consecutive No. 56, September, 1973

Generation time

Generation times ascribed for *V. parahaemolyticus* under optimum conditions are in the range of 8 to 14 min. Katoh (1965) observed that *V. parahaemolyticus* had a generation time of 11 to 13 min at 37°C, both on nutrient and fish extract liquid medium. An astonishingly short generation time of 7.6 min at the logarithmic growth phase was recorded by Aiso (1967) for *V. parahaemolyticus* cultured in brain-heart infusion broth (pH 7.9, supplemented with 1.5% NaCl) at 37°C. Ulitzur (1974) surmised that strains of *V. parahaemolyticus* could be categorized into two groups, one having a short generation time of 12

to 14 min at 37°C and the other with a longer generation time of 20 to 25 min at 37°C.

The extremely short generation time exhibited by *V. parahaemolyticus* under optimum temperatures is of distinct concern when considering its epidemiology. Assuming an average generation time of 10 min, over 10^6 cells could be produced from an initial number of only 10 cells in a matter of 3 hours (Nickelson, 1974). In most outbreaks of *V. parahaemolyticus* gastroenteritis, the incriminated food items were found to harbour relatively low counts of the pathogen originally but were subsequently stored at temperatures favouring proliferation of the bacterium. Such storage prior to consumption would permit *V. parahaemolyticus*, characterised by a short generation time, to attain hazardous levels. Lack of putrefactive appearance, unpalatable flavour or foul odour of fishery products implicated in *V. parahaemolyticus* food poisoning seem to be due to a quantitative rather than a qualitative difference, attributable to the much shorter generation time of *V. parahaemolyticus* as compared to other indigenous microflora and spoilage bacteria (Lee, 1973).

Inactivation in distilled water

Studies to determine the kinetics of *V. parahaemolyticus* inactivation in distilled water and other hypotonic solutions were initiated *a posteriori* to cognition of its halophilic nature. Yanagizawa (1964) and Lee (1972) demonstrated that *V. parahaemolyticus* was highly susceptible to suspension in distilled water. In the former report an exposure of less than 10 min yielded a 10^5 fold reduction in viable cells of *V. parahaemolyticus*, while in the latter, the exposure time required to inactivate 90% of the cells was only 0.9 to 4.4 min.

In view of such reports on the osmotic fragility of *V. parahaemolyticus*, it was presumed that washing of seafoods and kitchen utensils with tap water might prove to be an effective preventive measure of *V. parahaemolyticus* infection. However traces of salt and organic substances in freshwater may reduce the efficacy of this measure (Sakazaki, 1973). In fact Hidaka & Kakimoto

(1968) showed that the inactivation process was reversible provided the cells were returned to salt solution within 10 min.

Inactivation at elevated temperatures

Susceptibility of *V. parahaemolyticus* to inactivation at elevated temperatures is largely dependent on the cultural procedures used to grow the organism along with the type of menstruum in which the pathogen is harboured during the heat treatment (Beuchat, 1975). Vanderzant & Nickelson (1972) demonstrated that survival of *V. parahaemolyticus* in shrimp homogenate heated to 60 or 80°C for 15 min was determined by its initial population, the two being linked by a positive relationship. In contrast, regardless of the initial population, no survivors were detected in homogenates heated to 100°C for 1 or 5 min. Thermal inactivation times of 30, 15 and 5 min at 60°C have been reported by Liston *et al.* (1971), Sakazaki (1973) and Temmyo (1966) respectively. Further, Temmyo (1966), by simulating heating conditions normally encountered in processing plants, restaurants and homes, showed that *V. parahaemolyticus* could not be recovered from samples categorised as being "well cooked". In cases of *V. parahaemolyticus* gastroenteritis traced to consumption of cooked products, infection may therefore be attributed to insufficient heating or secondary contamination with subsequent storage at temperatures permitting multiplication of the pathogen.

Baab & Johnson (1974) and Goldmintz (1974) observed diphasic (fast followed by slower rates of death) thermal inactivation curves for several strains of *V. parahaemolyticus* heated at 47 to 49°C. A protective function against thermal inactivation of this halophilic bacterium seems to be afforded by the addition of sodium chloride to menstruum heated at 48°C (Covert & Woodburn, 1972; Goldmintz, 1974). Vanderzant *et al.* (1974) correlated the resuscitation efficacy of thermally stressed cells of *V. parahaemolyticus* to the level of sodium chloride in the recovery media.

Inactivation at low temperatures

Optimum temperatures reported for the growth of *V. parahaemolyticus* range from 35 to 37°C. The susceptibility of *V. parahaemolyticus* to low temperatures is well reflected in its seasonal abundance in natural environs during summer and its apparent "disappearance" during winter. While temperatures of 3 to 13°C have been shown to support growth of this organism, the lowest growth temperature in laboratory media was 5°C (Beuchat, 1973).

Matches *et al.* (1971) documented log reduction values of 2.2 to 6.2 in about 12 days for *V. parahaemolyticus* in fish homogenate stored at -18 and -34°C. Vanderzant & Nickelson (1972) reported that after an initial 2 log reduction within two days, the number of *V. parahaemolyticus* in inoculated whole, peeled and deveined shrimp held at 3, 7, 10 and -18°C remained relatively constant over the next six days.

Temmyo (1966), Liston *et al.* (1971) and Johnson *et al.* (1971) reported recovery of *V. parahaemolyticus* from frozen seafoods, thereby emphasizing the need for proper storage and terminal heating prior to consumption. The works of Matches *et al.* (1971) and Johnson & Liston (1973) indicate that *V. parahaemolyticus* on marine fish and shellfish is more susceptible to the lethal effects of chilling rather than to commercial freezing. However, *V. parahaemolyticus* seems to exhibit some degree of strain variability with regard to the effects of chilling and freezing on the survival of the pathogen in different foodstuffs held at the same temperature. Hence chilling or freezing *per se* cannot be relied upon to protect the consumer from seafoods which are subsequently exposed to conditions permitting outgrowth of this organism to hazardous levels (Liston, 1974).

Response to antibacterial agents

The relative efficiencies of various antibiotics, detergents, disinfectants and food preservatives against *V. parahaemolyticus* was extensively investigated by Yanagizawa (1967). The following is a list of some potent compounds inhibitory to *V. parahaemolyticus* (cited from Lee, 1973).

(a) 0.5 µg/ml chlorotetracycline (b) 0.05 to 0.1 mg/ml propyl-p-hydroxybenzoate (c) 30% glycerine (d) 12% commercially available sodium hypochlorite diluted with 3% sodium chloride to 1/3000th of its original concentration (e) 15% methyl or ethyl alcohol and (f) 0.5% hydrogen peroxide. Sanyal *et al.* (1973) tested the drug sensitivity of 111 strains of *V. parahaemolyticus* to several antibacterial agents and reported that all strains were sensitive to common antibiotics like streptomycin, tetracycline, neomycin, chloramphenicol, kanamycin, gentamycin and polymyxin B.

The antibiotic sensitivity pattern of 230 strains of *V. parahaemolyticus* (isolated from cases of gastroenteritis in Calcutta) was evaluated by Sen *et al.* (1977). Their results revealed that gentamycin and chloramphenicol inhibited 99.2% and 92.6% of the strains tested respectively. Doxycycline, neomycin and tetracycline were only moderately effective against the pathogen while ampicillin, kanamycin and streptomycin proved to be ineffective. Based on these findings Sen *et al.* (1977) recommended that chloramphenicol (equally effective against cholera vibrios), rather than the routinely prescribed tetracycline, should be the antibiotic of choice for treatment of *V. parahaemolyticus* induced human gastroenteritis.

Control measures

The ubiquitous distribution of *V. parahaemolyticus* in the marine milieu renders the protection of raw seafoods against contamination with this pathogen virtually out of question. An examination of 635 seafoods, comprising 30 marine species, by scientists of the Food and Drug Administration, U. S. A. (Fishbein *et al.*, 1974) revealed that over 70% of the samples tested harboured *V. parahaemolyticus*. However only 14% of the positive samples contained viable cells of *V. parahaemolyticus* at a level of 10³/g of seafood. The study indicated that naturally contaminated seafoods normally harbour low counts of 100 cells or less per gram.

V. parahaemolyticus is considered to be the only "causative organism" of seafood-borne illness, the cause as such being mishandling of the incriminated food

product (Nickelson, 1974). Most, if not all, outbreaks of gastroenteritis attributed to *V. parahaemolyticus* can be traced to seafoods with a history of unhygienic production, improper cooking, post-cooking contamination or inadequate refrigeration prior to consumption. Cross contamination through kitchen utensils, chopping boards and knives previously employed for seafoods may be responsible for cases of food poisoning wherein salted vegetables and other nonmarine food items have been implicated.

Under current technology it would seem quite impossible to suggest microbial standards for an organism that can be considered as part of the "natural flora" of freshly harvested seafoods since the high infective dose (10^6 to 10^9) of *V. parahaemolyticus* needed to produce illness would only undermine failure to comply with existing good manufacturing practices (Nickelson, 1974). Simple but strict hygienic measures to check multiplication of the pathogen in seafoods, proper thermal treatment and prevention of secondary contamination from raw seafoods appear to be the most tangible methods of control of infection in man (Sakazaki, 1973). Barrow (1974) aptly sums up the guidelines of control. According to him local culinary habits play a significant role in foodborne illness and food well-cooked shortly before consumption is always preferable. Since established customs die hard, safety is ultimately dependent not-so-much upon arbitrary microbiological standards, but on hygienic production, correct storage, proper distribution and most important of all, on the education of right eating habits.

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Estimation of Fishery Resources by Sonar Surveys

K. KRISHNA RAO*, S. NATARAJAN and G. P. EDWIN DANIEL

Pelagic Fishery Project, Cochin-682 016

The methodology adopted by the Pelagic Fishery Project, Cochin for surveying the surface fish schools with sonar equipment, is described. The estimates of the biomass arrived at from the surveys conducted during 1975-'78 are presented.

Estimation of fishery resources is important as the magnitude of the standing stock determines the fishing effort to be expended, as also the period and area for this purpose. In recent times electronic instruments are being extensively used in this estimation. Vessels fitted with the instruments scan the coasts to locate and estimate the different varieties of commercially important fishes. Echo integrator, echo sounder and scientific sonar are the acoustic instruments used in this endeavour. Of these, echo integrator along with echo sounder is used for estimating fish moving in lower strata, usually below 10 m to the bottom, while sonar is used for detecting the surface moving fish and estimating their abundance and echo sounder detects and records traces of moving fish well below the surface of the sea. Pelagic Fishery Project, Cochin has been making use of the above instruments and estimating the biomass of some of the important varieties of fish on the southwest and southeast coasts of India in different seasons since 1972. The details of estimates have been published in the various reports brought out by the Project (Anon 1974, '75, '76a, '76b). The method followed and the estimates arrived at in the course of recent surveys are presented in this communication.

Operation of sonar

The word sonar stands for sound navigation and ranging. It consists of transmitter, receiver, recorder, transducer with tilting and training mechanism and control cabinet. The block diagram of the operation of the sonar is given in Fig.1.

Transmitter is the main source of oscillation. This has an oscillator section which oscillates at ultrasonic frequency range and pulsed at a predetermined frequency, on receiving command from the recorder. This oscillator output is fed to the transducer which is normally fitted on to the keel of the vessel facing side ways, producing horizontal beam. It converts the electrical oscillation into mechanical vibration. These vibrations or sound waves travel horizontally in the water and get reflected by any object. The reflected sound waves are picked up by the same transducer which converts them into electrical energy. This reflected and received energy known as 'echo' is given to the receiver. It amplifies the received echo signal and feeds it to the recorder as voltage signal. Recorder has an electric pen running over a recording paper which makes a definite mark as and when an electrical signal passes through the pen. Thus a marking is displayed on receipt of echo signal on the recorder paper. Also the recorder marks when the transmitter transmits. The distance between the transmitter signal's mark and the received echo's mark is visually a measure of the time lag of the echo and is calibrated to read the range directly. Control cabinet enables us to control the parameters of the equipment like pulse length, band width, power output, beam width, range, training, tilting etc. Tilting mechanism is used to tilt the transducer to detect surface fish or deep water fish and training mechanism to rotate the transducer around 360° and to obtain the direction of the fish school with respect to the bow of the vessel.

The shape of the fish echo recorded on the paper depends on beam width, pulse length, range, tilt angle and paper speed. When the beam width is greater,

* Present address: Central Institute of Fisheries Technology, Cochin-682 029

the fish school is within the beam for a longer period giving the echotrace the shape of a bigger school. Also if two or more schools are separated by a small distance vertically, they will appear as one school on the recorder. Pulse length is having similar effect on the horizontal resolution. For the longitudinal length of the school (parallel to the track of the ship) the echogram for the same size of the school becomes smaller at short range and greater at long range as the beam is conical in shape. The range selector is changed from long range to short range when the school appears at a fixed distance and the paper speed is increased. The exactly opposite effect is produced when the range selector is changed from short range to long range. The echo picture is enlarged when the paper speed is increased and *vice versa*. Also for estimation of fish abundance the shape and dimension of the echo appearing on the recording paper

Frequency	: 24 KHz
Pulse length	: 4 milliseconds corresponding to 6 m
Tilt angle	: 0°
Beam angle (2A)	: 8°
Range	: 500 m
Training angle	: Fixed at 90° or 270° (Port or star board)

Method of estimation

In the acoustic surveys the vessel followed a grid of parallel tracks. A typical track is given in Fig. 2. The area surveyed was between 9°N on the east and 17°N on the west coasts.

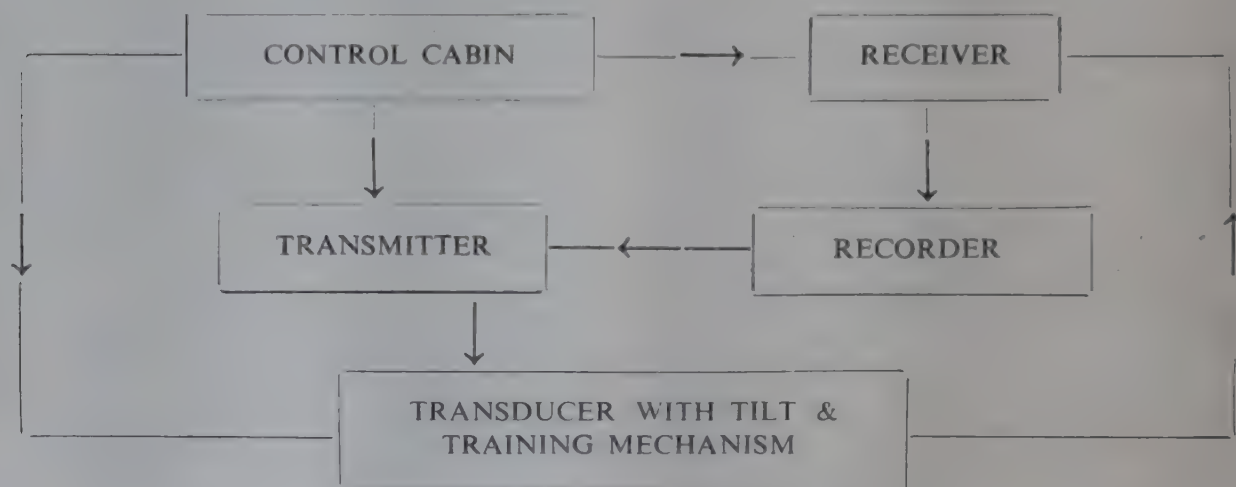


Fig. 1. Block diagram of sonar

is made use of. Hence the parameters affecting the shape of the echo should be controlled and accounted for estimation purposes. The sonar equipment used in R. V. Rastrelliger for the regular surveys of the Pelagic Fishery Project was Simrad Survey Sonar s.u. The parameters normally used during the sonar surveys are as follows:

After completion of a survey, the recorded paper from the sonar was suitably marked to tally with the areas of survey. Then the recordings on the paper pertaining to the schools of fish are isolated. The distance of the mid-point of the school from the vessel was measured and a frequency table of the number of schools in class intervals of 20 m was prepared. The extreme

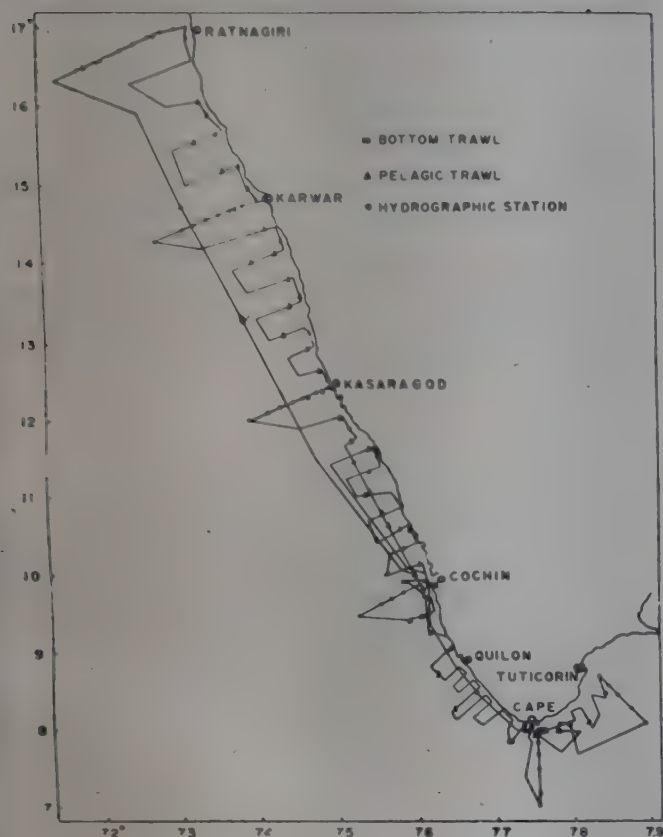


Fig. 2. Typical cruise track of the vessel

ends of the histograms show variations in the numbers of recorded schools from the rest, because of the difference in the effectiveness of the beam with the distance from the vessel. Deleting such extreme ones, the range over which the beam was effective, was determined which forms the basis for estimation of the number of schools along with track of the vessel. Thus if 20 to 220 m is found to be the effective range of the sonar and if 4 schools are recorded in one nautical mile of the track, the number of schools in one nautical mile square (one nautical mile along the track and one nautical mile across the track) is estimated by $4 \times \frac{1852}{200}$, that is, 37 schools, where 1852 is the number of metres in one nautical mile, 200 is the length of the effective range in metres.

The number of schools estimated in each square nautical mile was referred to as 'school density'. The estimated school densities were noted in each of the divisions of the vessel track in a chart covering the surveyed area. The division corresponding to a nautical mile, was referred to as

elementary sampling distance unit or briefly 'ESDU'. Boundary lines are drawn on the chart to distinguish the distribution areas of fish. The type of fish for the distribution areas (mackerel and oil sardine) was made out by visual observations of surface moving schools or from the fishing conducted by the vessel for identification of the traces. The distribution areas are further divided into four strata.

- Stratum 'a' : 0—50 schools per square nautical mile
- Stratum 'b' : 51—100 schools per square nautical mile
- Stratum 'c' : 101—200 schools per square nautical mile
- Stratum 'd' : 201 and more schools per square nautical mile

Fig. 3 shows distribution areas of some of the surface fish schools in one of the surveys.

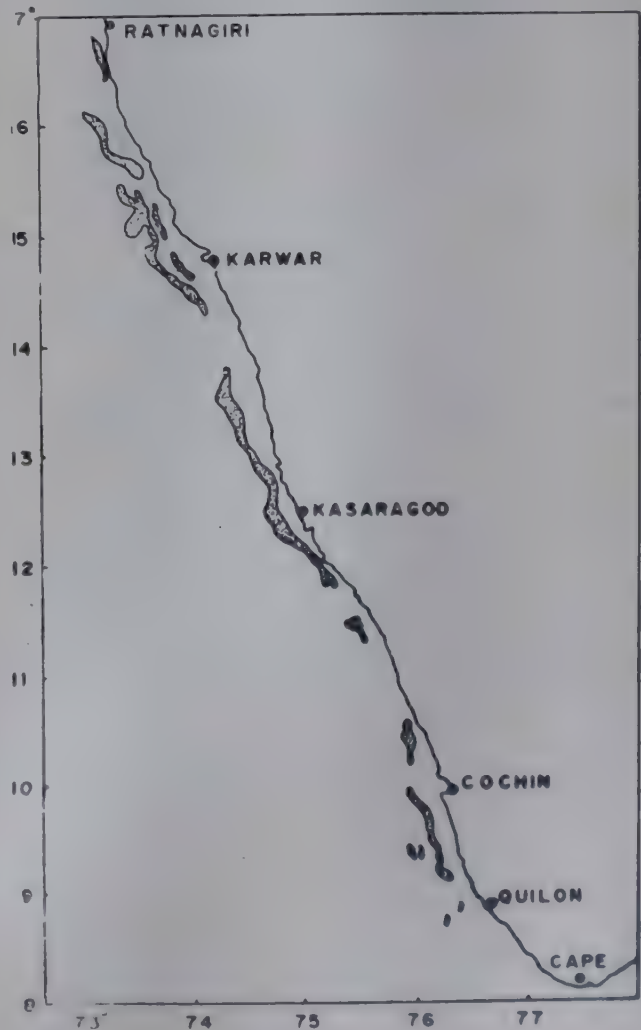


Fig. 3. Distribution of surface fish schools based on sonar observations (Sept. / Oct. 1975)

The estimations for biomass are done for 30' latitude intervals. The strata in each of the distribution areas, already mentioned were worked out separately for each 30' interval which is called a 'sub area'.

The schools covered within the effective range only were considered for the biomass estimation. Each of these schools was measured for the length axis of the school (L) parallel to the track of the ship, the breadth axis (B) perpendicular to the track of the ship and for the distance of the nearer end of the school from the vessel (D). The original measurements obtained from the sonar paper were converted into actual measurements. The apparent length of the school 'L', following Forbes & Nakken (1972), is given by $L = CS/I$, where C, is the speed of the paper in seconds per mm; S, the speed of the ship in metres per sec. and I, the measured width of target on chart in mm.

Breadth of the school was obtained from the width of the sonar paper corresponding to the range of the sonar. The true measurements of the dimensions of the school were obtained as follows:

$$L_c = L - 2 \left(D + \frac{B}{2} \right) \tan A$$

$$B_c = B - \text{Pulse length}/2$$

where 2A was the beam angle

The shape of the school was considered to be either elliptical or circular and the corresponding length and breadth of the school, L_c and B_c form the two axes of the ellipse in the former case, whereas in the latter the corrected breadth, B_c was the diameter.

The surface area of a schools of elliptical shape A_e is $\frac{\pi}{4} L_c B_c$ and for a school

of circular shape area A_c is $\frac{\pi}{4} B_c^2$. The

area of each stratum of distribution in a sub-area was measured by a planimeter and was converted to the actual area surveyed in square nautical miles by multiplying with a suitable raising factor. The area thus obtained was denoted by 's'.

The vertical extension of the school h, in the sub-area was estimated from the echo recordings. If $\bar{a}$ is the average number of schools in a stratum of the sub-area, the volume of school is $\bar{a} \bar{d} A_c h$ or $\bar{a} \bar{d} A_e h$ depending on the assumption of the shape of the school. The estimates of various strata from a sub-area were added up to obtain a single estimate for a sub-area. The first estimate of biomass, was thus obtained as total volume of schools in sub-area.

The packing density to convert volumes of schools to weight has been worked out on certain assumptions in each case separately. One method was to consider the distance between each fish in the school as one and a half times its length. In the surveys of 1975 and 1976, this procedure was adopted. A second method available was to consider the integrator deflection on the echo integrator for the surface schools recorded in the echo sounder. The integrator deflection was converted into total quantity and the volume of the surface school from echo recordings was obtained. By equating these two quantities, an estimate of the packing density was obtained. The method was followed in the surveys conducted in 1977 and 1978. As the packing densities differed in certain areas by this procedure, separate estimates of packing densities were obtained for different areas.

Sonar surveys

Sonar surveys have been conducted by the Project since 1975, mostly for the biomass estimates of mackerel and sardine. The surveys were arranged to cover the peak period of the fishing season. Only one survey each was conducted in 1975 and 1978 while in 1976 and 1977, 3 and 2 surveys were conducted respectively. The number of surveys in each year, the periods of the survey and the survey areas are given in Table 1.

The details of estimates are given in Tables 2 to 8. Though the original estimates were made for 30' interval, they are reproduced here for 1° interval.

Table 1. *Details of sonar surveys conducted by the Pelagic Fishery Project*

Year	No. of surveys	Period	Surveyed area
1975	1	September to October	17° to 8°
1976	3	(i) June to July	16° to 9°*
		(ii) July to August	16°30' to 8°30'*
		(iii) October	17° to 8°*
1977	2	(i) September	17° to 12° 45'
		(ii) November to December	17° to 9°*
1978	1	September to October	17° to 9°*

* Survey extended to east coast also

Table 3. *4/76-08 7 09 biomass (tonnes) latitude wise June to July 1976*

Latitude	Surface schools		Total
	Oil Sardine & mackerel	Others	
West coast			
17° to 16°	—	—	—
16° to 15°	2,691	0	2,691
15° to 14°	2,347	25	2,372
14° to 13°	1,210	0	1,210
13° to 12°	30,632	58	30,690
12° to 11°	125,731	762	126,493
11° to 10°	2,475	2,385	4,860
10° to 9°	15,077	2,085	17,162
9° to 8°	9,425	6,343	15,768
8° to 7°	0	3,181	3,181
East coast			
7° to 8°	0	6,142	6,142
8° to 9°	0	44,598	44,598
Total	189,588	65,579	255,167

Note: 1. — data not available
2. Biomass by sonar methodology from surface to 14 m depth

Table 2. *R/75-17 biomass (tonnes) latitude wise September to October 1975*

Latitude	Surface schools biomass
West coast	
17° to 16°	20,201
16° to 15°	159,758
15° to 14°	85,335
14° to 13°	240,374
13° to 12°	89,789
12° to 11°	54,148
11° to 10°	20,839
9° to 8°	72,662
8° to 7°	—
East coast	
7° to 8°	—
8° to 9°	—
Total	743,106

Note: — data not available

Table 4. *R/76-11 & 13 biomass (tonne)s latitude wise July to August 1976*

Latitude	Surface schools		Total
	Oil Sardine & mackerel	Others	
West coast			
16° 30' to 16°	1,024	247	1,271
16° to 15°	46,744	54	46,798
15° to 14°	201,764	7,261	209,025
14° to 13°	81,716	62,135	143,851
13° to 12°	111,715	3,101	114,816
12° to 11°	68,180	17,253	85,433
11° to 10°	67,459	10,703	78,162
10° to 9°	24,626	58,390	83,016
9° to 8°	321	0	321
8° to 7°	0	0	0
East coast			
7° to 8°	0	15,242	15,242
8° to 8°30'	0	643	643
Total	603,549	175,029	778,578

Note: Biomass by sonar methodology from surface to 14 m depth

Table 5. *R/76-16 biomass (tonnes) latitude wise October 1976*

Latitude	Surface schools		
	Oil Sardine & mackerel	Others	Total
West coast			
17° to 16°	78,838	44,168	123,006
16° to 15°	29,743	9,536	39,279
15° to 14°	24,068	0	24,068
14° to 13°	20,367	504	20,871
13° to 12°	15,556	4,763	20,319
12° to 11°	121,799	8,788	130,587
11° to 10°	27,657	7,610	35,267
10° to 9°	16,932	625	17,557
9° to 8°	15,097	1,197	16,294
8° to 7°	1,159	26,240	27,399
East coast			
7° to 8°	0	12,812	12,812
8° to 9°	—	—	—
Total	351,216	116,243	467,459

Note: 1. — data not available

2. Biomass by sonar methodology from surface to 17 m depth

Table 6. *R/77-13 biomass (tonnes) latitude wise September 1977*

Latitude	Surface schools	
	Oil Sardine & mackerel	Others
17° to 16°	15,998	—
16° to 15°	6,550	—
15° to 14°	3,915	—
14° to 13°	8,390	—
13° to 12° 45'	764	—
Total	35,617	—

Table 7. *R/77-14 biomass (tonnes) latitude wise November to December 1977*

Latitude	Surface schools	
	Oil Sardine & mackerel	Others
West coast		
17° to 16°	11,721	—
16° to 15°	7,034	—
15° to 14°	13,335	—
14° to 13°	1,124	—
13° to 12°	1,535	—
12° to 11°	333	—
11° to 10°	22	—
10° to 9°	119	—
9° to 8°	1,491	—
8° to 7°	169	—
East coast		
7° to 8°	31	—
8° to 9°	977	—
Total	37,891	—

Table 8. *R/78-15, 16 & 17 biomass (tonnes) latitude wise September to October 1978.*

Latitude	Surface schools		
	Oil sardine	Mackerel	Others
West coast			
17° to 16°	7,667	—	383
16° to 15°	11,054	—	—
15° to 14°	7,679	9,314	—
14° to 13°	59,065	16,474	4,783
13° to 12°	—	15,111	1,113
12° to 11°	1,944	12,140	769
11° to 10°	3,167	242	667
10° to 9°	—	2,554	2,064
9° to 8°	—	—	12,623
8° to 7°	—	—	948
East coast			
7° to 8°	—	—	2,038
8° to 9°	—	—	213
Total	90,576	55,835	25,601

The schools of mackerel and sardine could not be separated in some of the surveys as they occurred together. Besides, some other pelagic schools were also recorded by the sonar. These were mostly the schools of horse mackerel, tuna and lesser sardines and the estimates were given in the Tables 2 to 8. The estimate of combined stock of mackerel and oil sardine was 700 thousand tonnes in 1975. In 1976 the three surveys gave estimates varying between 190 and 600 thousand tonnes. In 1978, sardines were estimated at 91,000 and mackerel 56,000 tonnes. The above shows high fluctuations in the biomass. The estimates are to be considered as applicable for the period of surveys. They undergo tremendous variation over the period. Past experience is an indication in the matter of mackerel and oil sardine fishery in this respect. The surveys can be made at suitable periods of time so that the estimates of biomass can be utilised for a proper exploitation of the resources. With proper coordination between the different agencies, the sonar surveys can be taken advantage of, for augmenting the fishing effort to obtain improved catch.

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Eradication of Uneconomical Fishes with Simple Gill Nets at Hirakud Reservoir

A. A. KHAN, V. C. GEORGE and M. D. VARGHESE

Burla Research Centre of Central Institute of Fisheries Technology, Burla-768 017

Experiments with simple gill nets of mesh bar 25, 30, 35, 40 and 45 mm were carried out to determine the suitable mesh size for the eradication of the uneconomical fishes of Hirakud reservoir. Results show that net with 25 mm bar is more suitable particularly for *Gudusia chapra* (Ham), *Rohtee cotio* (Day) and *Eutropichthys vacha* (Ham).

Selective stocking of the desirable species or elimination of undesirable species is important for the management of reservoir fisheries (Lepitzky, 1965; Anon, 1976). The fishes other than desirable species have been classified as uneconomical fishes, undesirable fishes, trash fishes, weed fishes and minnows (Natarajan, 1976). The influence of these fishes on reservoir fishery has been discussed by Bennet (1962), David *et al.* (1969), Shetty (1969), Natarajan (1972 a, b) and Jhingran (1977). Jhingran (1977) while discussing the regulation of fish stock in the reservoirs of U.S.S.R. has suggested eradication of these fishes at the pre-impoundment stage either by selective fishing or through specific predatory fishes. Ali-kunhi (1971) has recommended better utilisation of these fishes. Natarajan *et al.* (1971) and David *et al.* (1969) have suggested removal of peripheral trees for the effective operation of gears for eradicating trash fishes. Job *et al.* (1955) found that, out of 86 species in Mahanadi, 62 were uneconomical. Except that of Znamensky (1967) systematic attempts to eradicate uneconomical fishes are lacking. This paper reports the authors' attempts to remove *G. chapra*, *R. cotio* and *E. vacha* from Hirakud reservoir by gill netting.

Materials and Methods

Simple gill nets of mesh bar 25, 30, 35, 40 and 45 mm were used for this investigation as detailed in Table 1. The nets were operated as surface set in the evening and hauled up next day morning. The

nets were operated parallel and perpendicular to the shore and the positions interchanged giving equal chances to all nets. Day to day species wise catch and the morphometry of fish caught from September 1977 to May '78 were recorded (Tables 2 and 3).

Results and Discussion

As evident from Tables 2 and 3, the nets of 25 mm bar were found most effective followed by 30 and 35 mm bar nets. 25 mm bar net caught 2.716, 7.965, 16.520 and 27.534 times more by weight compared to those of 30, 35, 40 and 45 bar nets respectively. The predominant species were *G. chapra*, *R. cotio* and *E. vacha* of size ranges 141–200, 101–220 and 221–280 mm respectively.

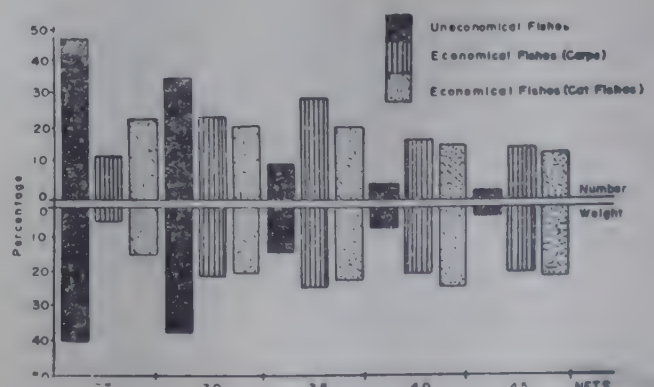


Fig. 1. Percentage catch in number and weight of economical and uneconomical fishes

Table 1. *Specification of nets*

Mesh bar mm	Material	Type of knot	Twine size	Length	Depth	Vertical	Hanging coefficient	Selvedge	Head and foot rope	Floats	Sinkers
25			210/1/3	1400	70			2 mesh in		Alumi- nium,	Mild steel
30		Double	210/1/3	1166	58			depth, both	Nylon	75 mm Ø	ring type
35	Nylon	trawl	210/1/3	1000	50	0.86	0.50	in upper and	rope,	spherical,	weighing
40		knot	210/2/2	875	44			lower edges	3 mm Ø	5 each	100 g,
45			210/2/2	778	39			with 210/2/2 winet			5 each

Table 2. Catch composition

Uneconomical fishes	25 mm bar net			30 mm bar net			35 mm bar net			40 mm bar net			45 mm bar net			Per-cent
	Num-ber	Per-cent	Wei-ght kg	Num-ber	Per-cent	Wei-ght kg	Num-ber	Per-cent	Wei-ght kg	Num-ber	Per-cent	Wei-ght kg	Num-ber	Per-cent	Wei-ght kg	
<i>G. chapra</i>	1580	72.25	71.03	1041	61.60	55.78	298	51.73	13.94	19.46	46.86	6.17	76	44.70	3.60	8.00
<i>R. cotio</i>	292	13.36	17.82	338	20.00	20.80	67	11.63	4.46	6.23	3.70	0.80	4	2.36	0.25	0.55
<i>E. vacha</i>	183	8.37	22.58	165	9.76	26.17	86	14.93	16.60	23.16	15.50	9.90	20	11.76	6.40	14.23
<i>C. reba</i>	9	0.41	0.85	10	0.76	1.70	7	1.21	1.20	1.70	0.73	0.45	—	—	—	—
<i>B. sarana</i>	9	0.41	0.66	5	0.30	0.50	2	0.34	0.30	0.42	1.10	0.60	1	—	—	—
<i>R. chrysea</i>	2	0.09	0.74	2	0.11	0.15	2	0.34	0.15	0.20	0.38	0.10	—	—	—	—
Economical fishes																
<i>C. catla</i>	—	—	—	2	0.11	0.15	1	0.17	0.20	0.27	—	—	1	0.58	0.20	0.44
<i>L. rohita</i>	—	—	—	—	—	—	2	0.34	0.40	0.55	1	0.38	—	—	—	—
<i>L. bata</i>	4	0.18	0.28	2	0.11	0.20	8	1.38	1.36	1.90	4	1.47	1	0.58	0.20	0.44
<i>L. fimbriatus</i>	—	—	—	5	0.30	0.55	2	0.34	0.70	0.98	1	0.38	—	—	—	—
<i>L. calbasu</i>	6	0.27	0.62	10	0.60	3.50	11	1.90	2.40	3.35	8	2.98	10	5.90	5.75	12.79
Cat fishes																
<i>S. silondia</i>	98	4.48	17.85	99	5.96	23.92	80	13.88	26.75	37.32	64	23.62	51	30.00	23.55	52.39
<i>P. pangasius</i>	2	0.09	0.50	5	0.30	1.20	1	0.17	0.10	0.13	—	—	—	—	—	—
<i>M. seenghala</i>	2	0.09	0.90	2	0.14	0.55	3	0.52	1.45	2.03	3	1.10	5	2.95	4.80	10.67
<i>M. aor</i>	—	—	—	4	0.24	1.65	5	0.86	1.45	2.03	2	0.73	—	—	—	—
<i>W. attu</i>	—	—	—	—	—	—	1	0.17	0.20	0.27	3	1.10	1	0.58	0.20	0.44

Table 3. Catch of predominant fishes in various nets

Fishes	25 mm bar net		30 mm bar net		35 mm bar net		40 mm bar net		45 mm bar net	
	Number	Weight kg	Number	Weight kg	Number	Weight kg	Number	Weight kg	Number	Weight kg
<i>G. chapra</i>	1580	71.035	1041	55.78	298	13.94	127	6.175	76	3.60
<i>R. cotio</i>	292	17.820	338	20.80	67	4.46	10	0.800	4	0.25
<i>E. vacha</i>	183	22.580	165	26.17	86	16.60	42	9.900	20	6.40
Total	2055	111.435	1544	102.75	451	35.00	179	16.875	100	10.25
Total area of net operated in sq. m	18480		46200		46200		46200		46200	
Catch/1000 sq. m kg	6.030		2.220		0.757		0.365		0.221	

Table 4. *Analysis of variance*

Source	ss	df	ms	f
Total	56.7891	399		
Between nets	18.1295	4	4.5324	112.47***
Between days	25.9215	79	0.3281	8.14***
Error	12.7381	316	0.0403	

*** Significant at 0.1% level

Statistical analysis of the data (Table 4) showed that 'between nets' and 'between day' variations were highly significant ($P < 0.001$). The least significant difference at 5% level for 25 mm bar net was worked out to be 0.0620. The mean catches of the nets of 25, 30, 35, 40 and 45 mm bar were respectively 0.6967, 0.4860, 0.2386, 0.1692 and 0.1492 kg showing higher catch by 25 mm bar net. Further the 25 mm bar net caught the least number of economic fishes (Fig. 1) compared to other nets establishing its suitability over others.

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Iced and Frozen Storage Characteristics of Cultured *Chanos Chanos* (FORSKAL)

JOSE JOSEPH, P. A. PERIGREEN, CHINNAMMA GEORGE and T. K. GOVINDAN
Central Institute of Fisheries Technology, Cochin-682 029

Freshly harvested milk fish (*Chanos chanos*) were stored in crushed ice and their storage life estimated by following biochemical, bacteriological and organoleptic changes occurring during storage. Samples of the fish were withdrawn at various intervals of storage, quick frozen, glazed and held in frozen storage at -18°C. Shelf-life in frozen storage was determined in relation to period of ice storage prior to freezing by determining biochemical and organoleptic characteristics upto 30 weeks.

Milk fish, *Chanos chanos* (Forsk.) occur throughout the Indo-Pacific area. The adult fish generally live and spawn in the open sea. But their fry, fingerlings and even juveniles are found in the brackish water regions. Even though milk fish do not form a significant marine fishery, they constitute a good estuarine fishery. They are also one of the most suitable species for brackish water fish culture because of their vast adaptive powers to wide variations in the living medium, abundant occurrence and fast growth. They are widely spread in peninsular India from Orissa and Andhra coasts in the east to Karnataka coast in the west and around Andaman and Lakshadweep.

Out of the nearly 2 million hectares of brackish waters available in India, 2.6 lakhs hectares could be converted into salt water fish ponds, even though at present only a very insignificant area is being utilized as well-organised fish ponds. It is estimated that 800 million *Chanos* fry can be successfully reared in a year if all the culturable area mentioned above is utilized (Tampi, 1973). This works out to a potential yield of 4 lakh tonnes of *chanos* per annum, assuming that the fish grows to an average weight of 500 g.

Such increases in the resources of the fish call for development of proper post-harvest technology so that the fish is put to maximum economic utility. Frozen storage studies of fresh water fishes like *Labeo rohita*, *Catla catla*, *Cirrhina mrigala*, *Labeo calbasu*, *Mystus seenghala* and *Wallago attu* have been

reported by Devadasan *et al.* (1978). Factors affecting keeping quality of *Barbus carnaticus*, *Barbus dubius*, *Labeo sp.*, *Ophiocephalus* and *Wallago attu* (Lahiri *et al.*, 1963), *C. mrigala* (Nair *et al.*, 1971) and *C. catla*, *L. rohita*, *C. mrigala*, *L. calbasu* and *B. carnaticus* (Nair *et al.*, 1974) during chilled storage have also been studied. But so far no research has been carried out on the preservation of our major brackish water fishes. Hence it was thought of interest to study the proximate composition, iced and frozen storage characteristics of *Chanos chanos* (Forsk.), a variety widely cultured in all brackish water fish farms as well as occurring in large quantities in the back-waters, estuaries and brackish water lakes of the country.

Materials and Methods

Chanos chanos, freshly harvested from the Narakkal brackish water fish farm of the Central Marine Fisheries Research Institute, Cochin, were iced immediately and brought to the laboratory within four hours of removal from water. The fish weighed 809 g on an average and were used in these studies after cleaning in tap water. The muscle was analysed in the fresh condition for moisture, crude proteins, fat, ash, acid insolubles and non-protein nitrogen (NPN) by the methods of AOAC (1955). Sarcoplasmic and myofibrillar proteins were determined by the method of King (1966), prolamine by the method of Green & Hughes (1962), stroma proteins by the method of Sayre (1968), sodium, potassium and calcium by flame photometry

(Vogel, 1961), alpha amino nitrogen by the method of Pope & Stevens (1939), ribose by Mejbam's method (1939), fructose by method of Rae (1934), glycogen by method of Kleiy (1951) and phosphorus by the method of Fiske & Subba Row (1925).

The whole fish were stored in an insulated box in crushed ice in 1:1 proportion with replenishment of ice. Samples were drawn at intervals of 0, 4, 7, 10, 14 and 18 days and analysed for moisture, total nitrogen (TN), NPN, peroxide value (PV), salt soluble nitrogen (SSN), standard plate count (SPC) and overall organoleptic quality. On each day of sampling, representative samples were quick frozen, glazed, packed in polythene sheets and stored at -18°C. Samples from frozen storage were drawn and analysed at intervals of 2, 11, 20 and 30 weeks for moisture, TN, NPN, SSN, PV and organoleptic quality.

Table 1. *Proximate composition and protein fractionation of the fresh muscle*

Moisture %	:	71.69
Crude protein (TN x 6.25) %	:	24.06
Proteins % (TN-NPN)x 6.25	:	20.47
Sarcoplasmic proteins (% of proteins)	:	34.52
Myofibrillar proteins (% of proteins)	:	52.36
Prolamines (% of proteins)	:	1.96
Stroma protein %	:	0.71
Non-protein nitrogen mg/100 g	:	560
Free alpha amino nitrogen mg/100 g	:	140
Ether extractables % (O.W.B.)	:	3.510
Ash % (O.W.B.)	:	1.460
Acid insolubles % (O.W.B.)	:	0.046
Potassium mg/100 g	(O.W.B.) :	307
Sodium mg/100g	(O.W.B.) :	61
Calcium mg/100 g	(O.W.B.) :	190
Ribose mg/100g	(O.W.B.) :	559
Fructose mg/100 g	(O.W.B.) :	12
Glycogen mg/100 g	(O.W.B.) :	117
Phosphorus mg/100 g	(O.W.B.) :	202

Results and Discussion

Results of analyses of the fresh fish muscle are presented in Table 1. Changes occurring in the fish muscle during ice storage are shown in Table 2. Moisture content gradually increased from 71.69 to 73.64% in the course of 18 days. TN showed a gradual decrease from 3.726 to 3.418%. This is in full agreement with the trend observed in all the references cited earlier. Organoleptically the fish were in excellent to good condition for 4 days and in fair condition for 10 to 14 days in ice, after which the eating quality deteriorated considerably. Toughening of texture and loss of juiciness and characteristic flavour were the main symptoms of deterioration. Of course, the fish were on the border line of acceptability for about another week, though definitely much inferior in quality and hence not at all comparable with those stored in ice for less than two weeks.

Changes in moisture contents of the muscle during frozen storage are shown in Table 3. The trend is the same as observed in the case of the other fishes studied earlier, that is, a gradual decrease along with increase in storage period. The changes in TN during frozen storage (Table 4) are not significant. Changes in NPN (Table 5) during frozen storage show some interesting trend. In general, an increase is observed upto 11 weeks of storage with a drastic fall when analysed in the 20th week. This fall may be attributed to the drip loss while thawing due to prolonged frozen storage, which invariably occurs in such cases. Changes in SSN during frozen storage are presented in Table 6. Gradual fall in these values are observed along with storage period due to denaturation of proteins. Table 7 indicates the changes in PV during frozen storage. As is to be expected, a gradual increase in the values is observed during frozen storage due to oxidation of the fat. Organoleptic changes occurring in the fish muscle during frozen storage are presented in Table 8. The broad observations are that the frozen storage life of the freshly frozen fish is 30 weeks, that of fish stored in ice for 4 days prior to freezing is 20 weeks, that of fish held in ice for 7 days and then frozen is 11 weeks, that of fish stored in ice for 10 days before freezing is 2 week and that of

Table 2. *Changes in muscle during storage in ice*

Storage period days	Moisture %	TN %	NPN %	PV ml N/500 thio/g fat	SSN % of TN	SPC/g	Overall quality
0	71.69	3.726	0.560	0	67.57	764	Excellent
4	72.01	3.616	0.532	10.86	65.47	11,330	Good
7	72.45	3.584	0.504	12.32	64.62	97,970	Fair to good
10	72.21	3.506	0.560	12.14	61.94	252,300	Fair
14	72.68	3.520	0.588	23.88	60.64	55,630	Fair
18	73.64	3.418	0.560	22.12	58.16	—	Fair to poor

Table 3. *Changes in percentage moisture contents during frozen storage*

No. of days in ice before freezing	Initial value (before freezing)	Period of frozen storage in weeks			
		2	11	20	30
0	71.69	71.30	71.23	70.92	70.82
4	72.01	71.10	70.18	70.01	70.23
7	72.45	71.89	70.94	71.21	70.89
10	72.21	71.17	71.16	70.85	71.08
14	72.68	72.23	71.52	70.12	—
18	73.64	—	—	—	—

Table 4. *Percentage change in total nitrogen contents during frozen storage*

No. of days in ice before freezing	Initial value (before freezing)	Period of frozen storage in weeks			
		2	11	20	30
0	3.726	3.780	3.612	3.706	3.672
4	3.616	3.790	3.558	3.631	3.605
7	3.584	3.504	3.536	3.712	3.482
10	3.506	3.613	3.436	3.501	3.382
14	3.520	3.413	3.591	3.602	3.435
18	3.418	3.502	3.282	3.481	3.372

Table 5. *Percentage changes in non-protein nitrogen contents during frozen storage*

No. of days in ice before freezing	Initial value (before freezing)	Period of frozen storage in weeks			
		2	11	20	30
0	0.560	0.610	0.752	0.488	0.5282
4	0.532	0.506	0.821	0.392	0.4106
7	0.504	0.701	0.662	0.336	0.3914
10	0.560	0.656	0.608	0.426	0.4118
14	0.588	0.412	0.524	0.492	0.4804
18	0.560	—	—	—	—

Table 6. *Percentage changes in salt soluble nitrogen (% of total nitrogen) contents during frozen storage*

No. of days in ice before freezing	Initial value (before freezing)	Period of frozen storage in weeks			
		2	11	20	30
0	67.57	64.27	63.39	61.40	50.34
4	65.48	58.15	62.79	58.16	47.37
7	64.62	62.22	57.91	49.56	46.35
10	61.94	59.56	56.22	53.64	—
14	60.44	52.81	48.16	—	—
18	58.16	—	—	—	—

Table 7. *Changes in peroxide value (ml N/500 thiosulphate/g of fat) in muscle during frozen storage*

No. of days in ice before freezing	Initial value (before freezing)	Period of frozen storage in weeks			
		2	11	20	30
0	0	12.82	17.18	18.12	26.28
4	10.86	15.78	21.52	...	24.12
7	12.32	18.24	33.19	26.15	23.82
10	12.14	16.80	26.15	31.15	22.11
14	23.88	20.50	56.38	...	...
18	22.12	38.16	...	...	...

Table 8. *Changes in organoleptic characteristics during frozen storage*

No. of days in ice before freezing	Initial quality	Period of frozen storage in weeks			
		2	11	20	30
0	Excellent	Good	Good	Fair	Fair to poor, acceptable, less juicy
4	Good	Good	Good	Fair	Poor
7	Fair to good	Fair to good	Fair	Poor	Poor
10	Fair	Fair	Poor	...	...
14	Fair	Fair to poor, acceptable	Poor, not juicy, rancid	...	...
18	Fair to poor	poor	...	...	...

fish held in ice for 14 days prior to freezing is less than 2 weeks. Border lines of acceptability are reached after these time intervals in frozen storage. Though the fish do not become totally unacceptable after these periods, eating qualities are adversely affected. Thus *Chanos chanos*, freshly

harvested from fish farms could be held in edible condition in crushed ice for 12 to 14 days. The fish held in ice for different periods ranging from 0 to 14 days and then frozen, showed shelf lives of less than 2 to 30 weeks at -18°C in an inverse relation to the period of storage of the fresh fish in ice.

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Utilization of Frog Waste

A. LEKSHMY NAIR and P. V. PRABHU

Central Institute of Fisheries Technology, Cochin-682 029.

Commercial frog waste samples have been converted into meals by cooking at 0.7 kg/sq. cm for 30 min, draining off the stick water and drying the press cake either in the sun, tunnel dryer under controlled conditions or hot air oven. Yield of the meal varied between 18.6 to 21.5 per cent of the fresh frog waste. Chemical analyses of the meals have shown that the meals prepared from frog waste conform to standards prescribed for fish meal and livestock feed and can therefore be used for supplementation of poultry/animal feed.

Hind legs of certain varieties of frogs are an item of luxury food and a delicacy in western countries. Frog meat is also consumed by the poor and tribal people in certain other parts of the world. The common edible species available in India and mainly distributed in Kerala, Tamil Nadu, Assam, Orissa, West Bengal and U. P. are *Rana hexadactyla* LESSON, *Rana trigrina* DAUD and *Rana trigrina* CRASSA-JERD. Although the industry in the initial stages was mainly concentrated in and around Cochin, it has now been established in different parts of the country. Total export of frozen frog legs from India has shown a steady increase from 840 tonnes valued at Rs 1.18 crores in 1969 to 3510 tonnes valued at Rs. 8.42 crores in 1978 (Anon, 1979). Since only hind legs of frogs are used for processing, the front legs and other body portions are disposed off as waste. On an average these portions constitute about 65 per cent by weight of the whole frog. Considering the vast quantity of frozen frog legs exported at present, about 5400 tonnes of frog wastes are discarded annually on an average. If proper methods of utilization of this waste could be developed, it would add significantly to the returns from the industry as well as help in solving in part the protein shortage in the country.

Reports on the quality characteristics of frog legs or frog waste are limited. The proximate composition and nutritive value of leg meat of two edible species of frogs *R. hexadactyla* and *R. trigrina* have been reported by Dani *et al.* (1966). Rao & Kamasastri (1963) have reported the yield

of oil and meals prepared by the wet reduction and dry reduction methods from the waste material in the frog leg processing industry, their chemical composition and storage changes. The present communication is a report of the investigations carried out by the authors to evolve suitable methods for proper utilization of the wastes from the frog leg processing industry.

Materials and Methods

Frog waste was collected from various frog cutting centres near Cochin. This was washed well with water to remove dirt and other extraneous matter sticking to the material and processed either immediately or frozen and stored at -25°C until taken out for further processing. Live frogs were also procured, the hind legs cut off according to the method of Iyer & Chaudhuri (1968) and the residual waste used for extraction of oil and preparation of meal. The raw waste was cooked in steam at 0.7 kg/sq. cm for 30 min, the stick water was drained off and the press cake dried in the sun for 20–24 h or in a tunnel dryer under controlled conditions at $45\text{--}50^{\circ}\text{C}$. To study the effect of different types of drying on the nutritive quality of the frog meals, the cooked residue from the same batch was dried (i) in the sun (ii) in tunnel dryer at 50°C (iii) in air oven at 70°C and (iv) in air oven at 98 to 100°C for 24, 18, 14 and 10 h respectively. The material was turned from time to time to prevent scorching during the drying process. The dried product was powdered in a mechanical pulveriser, sieved through 40 mesh sieve and analysed. The

meals were stored in bakelite capped glass bottles at room temperature for storage studies.

Moisture, fat, crude protein, ash and acid insoluble ash were estimated according to AOAC (1975). Total volatile bases were determined by distillation of the trichloroacetic acid extract with saturated sodium borate, followed by absorption in boric acid and alpha amino nitrogen by the method of Pope & Stevens (1939). The ash was dissolved in 1 N hydrochloric acid and used for the estimation of calcium using Systronics flame photometer and phosphorus by the method of Fiske & SubbaRow (1925). Estimation of available lysine was carried out according to the modified Carpenter procedure (Booth, 1971). Amino acid composition was determined by standard microbiological assay methods (Shockman, 1963).

Results and Discussion

The yield of frog meal was found to range from 18.6 to 21.5% of the original fresh weight of the waste in the various experiments. The results of chemical analyses of five frog meal samples prepared from commercial waste are presented in Table 1. The crude protein value in the different meals ranged from 62.6 to 72.3% with ash ranging from 14.7 to 22.6%. The acid insoluble ash varied between 0.3 and 0.5%, the fat between 6.8 to 12.3% and the available lysine from 7.4 to 8.1 g/16 g N₂ in the various samples tested. This is in accordance with the chemical characteristics

of the meal prepared by wet reduction process by Rao & Kamasastri (1963) except for the available lysine. Such variations in the quality of commercial fish meal samples have been observed by Mac Intyre (1957), Srinivasan (1966) and Mathen *et al.* (1975). Factors like differences in the size and quality of the initial raw waste, the season at which the meals are prepared and varying degrees of removal of stick water during preparation of the meal probably accounted for such wide fluctuations in the crude protein of the samples prepared from commercial waste. Samples prepared from waste obtained after removal of legs in the laboratory showed a more uniform pattern in the chemical indices (Table 2). Usually fish meal is graded on its protein content. The prescribed maximum limit for moisture in fish meals is 10%, for fat and acid insoluble ash 10% and 5% respectively and the minimum limit for crude protein 50% according to the relevant specifications (IS: 4307-1973). Except for one sample (Table 1) which showed a higher fat content, all samples tested conformed to the prescribed standards. The excessive fat in this sample may have been due to incomplete pressing and removal of stick water from the cooked waste. Recent studies have disproved the belief that growth was depressed in chicks fed on fish meals of high fat and have shown that poultry can utilise fat efficiently, provided the diet has the correct ratio of protein to calorific value and is adequately fortified with vitamins (March, 1962).

Table 1. Composition of frog waste meal from commercial waste

	Samples				
	1	2	3	4	5
Moisture %	8.3	6.5	6.2	6.5	7.4
*Crude protein % (TN x 6.25)	72.3	67.4	62.6	66.2	65.8
*Fat %	12.3	10.8	10.8	8.2	6.8
*Total ash %	14.7	18.4	22.6	21.8	22.4
*Acid insoluble ash %	0.4	0.3	0.5	0.4	0.4
TVN mg/100 g	26.1	29.0	27.7	24.8	26.6
Alpha amino N mg/100 g	50.1	52.5	48.8	48.8	50.5
*CaO %	6.2	7.1	6.2	7.8	8.0
*P ₂ O ₅ %	5.4	6.4	5.4	6.5	5.8
Available lysine g/16 g N ₂	8.1	7.6	7.6	7.4	7.6

*Moisture free basis

Table 2 summarises the analytical data of meals prepared by using various methods of drying. Meals dried in the sun and tunnel dryer have higher moisture content and TVN values, but the alpha amino nitrogen and available lysine are nearly the same in all the four meals. The loss in available lysine during hot air drying ranged from 0.2 to 0.3 g/16 g of N₂, showing that the decomposition during the drying process is not very significant. Rao *et al.* (1965) have also reported similar observations during their studies on nutritive value of proteins in fish meals. A loss of 0.6 to 0.7 g of available lysine per 16 g of N₂ was observed during storage of the frog meal for 10 months at room temperature.

It may be seen from Table 3 that the amino acid composition of the frog meal compares very favourably with that of fish meals prepared from sardine and miscellaneous fish (M. A. James, Central Institute of Fisheries Technology, Cochin—personal communication) and also silver belly (Srinivasan, 1966). With careful method of processing, meals from frog waste can therefore be used as a good substitute for fish meals for supplementation of poultry/animal feed. Recent work of Sulthan *et al.* (1980) has shown that the use of an artificial feed compounded by using frog flesh waste, groundnut oil cake, rice bran, molasses, seaweed powder, sun dried shrimp head meal, urea, polyphosphate and a few drops of acetic acid in definite proportions in the

Table 2. *Effect of different types of drying on composition of frog waste meals*

	Mode of drying			
	Sun	Tunnel	Hot air at 70°C	98–100°C
Moisture %	8.5	5.4	3.6	5.3
*Crude protein % (TN x 6.25)	67.1	67.5	68.3	67.3
*Fat %	7.6	7.1	7.5	7.2
*Total ash %	21.8	22.3	21.3	21.8
*Acid insoluble ash %	0.4	0.4	0.3	0.3
TVN mg/100 g	28.8	29.0	26.4	25.0
Alpha amino N mg/100 g	50.2	52.5	51.8	50.6
Available lysine g/16g N ₂	8.0	8.1	7.9	7.8
Available lysine after storage for 10 months g/16g N ₂	7.4	—	7.2	—

*Moisture free basis

Table 3. *Amino acids of frog meal and fish meal samples*

Amino acids	Frog		Sardine	Miscellaneous fish			Silver belly
	1	2		1	2	3	
Arginine	4.1	5.1	6.1	5.9	5.8	6.4	5.2
Histidine	2.4	2.3	3.1	1.8	1.8	2.0	2.4
Lysine	7.8	7.2	7.9	6.2	5.8	6.9	7.7
Tyrosine	2.9	2.4	3.1	2.6	2.6	2.8	—
Tryptophan	0.7	0.8	0.9	0.8	0.7	0.8	0.8
Phenylalanine	4.4	4.9	2.8	2.8	2.9	3.1	3.5
Methionine	2.4	3.2	2.4	2.3	2.1	2.8	2.2
Cystine	0.4	0.4	1.3	0.8	0.9	0.8	—
Threonine	3.4	2.9	3.8	3.1	2.8	3.5	2.9
Leucine	7.4	6.4	6.9	6.2	6.2	6.4	5.2
Isoleucine	5.3	5.2	4.1	3.6	3.4	3.7	4.6
Valine	3.8	4.2	4.9	5.0	4.9	5.1	4.2
Glutamic acid	11.6	10.4	11.4	12.4	11.9	12.7	—
Glycine	2.3	2.9	4.8	4.4	3.0	4.7	—

form of pellets gave encouraging results in the growth and survival of cage cultured prawns. The feed proved to be nutritious with high conversion value and was quite acceptable.

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Freezing and Cold Storage of Cat Fish Fillets

P. A. PERIGREEN and JOSE JOSEPH

Central Institute of Fisheries Technology, Cochin-682 029

The freezing and cold storage changes occurring in skinless fillets of cat fish and the effect of packaging on the quality of frozen fillets during storage at -18°C were studied. Maximum shelf-life of 27 weeks was shown by fillets frozen as glazed (water) blocks and packed in polythene lined waxed cartons.

Cat fishes constitute one of the important fisheries of India, the average annual landings for the years 1971-'78 being 53,138 metric tonnes, accounting for 4.2% of the total marine fish landings. This is an unpopular variety of fish fetching very low price in our markets. It is not liked by many people in round form, mainly due to its unattractive appearance and peculiar smell. Filleting is one of the methods of improving the consumer appeal of such varieties of fishes. The technological aspects of production of fillets from different varieties of fishes have already been worked out (Perigreen *et al.*, 1979). The freezing and storage characteristics of fillets of seer fish have been studied by Shenoy (1976). Devadasan *et al.* (1978) studied the freezing and storage characteristics of fillets from six species of fresh water fishes. This paper reports the freezing and cold storage changes in skinless fillets of cat fish (*Tachysurus* sp.) and the effect of packaging on the quality of frozen fillets during storage at -18°C .

Materials and Methods

Cat fish were procured from landing places at Fort Cochin, brought to the laboratory immediately, washed well and stored in crushed ice. The fish were then separated into different size groups and skinless fillets were prepared (Perigreen *et al.*, 1979). Both filleting and skinning were done manually. The belly flaps and fins were removed from the fillets which were then thoroughly washed in potable water and drained for about 15 minutes. The yield of skinless fillets was noted. The fillets were then frozen in contact

plate freezer at -35 to -40°C and stored as follows.

- *1. Fillets frozen individually
- *2. Individual fillets wrapped in polythene sheet (200 gauge) and frozen
- *3. Fillets packed in plain waxed cartons (400 g each) and frozen as blocks
- *4. Fillets packed in waxed cartons (400 g each) lined inside with polythene sheet (200 gauge) and frozen as blocks
- *5. Fillets packed in waxed cartons (400 g each) lined inside with polythene (200 gauge), glaze water added to cover the fillets completely and frozen as blocks

After freezing, all the samples were stored at -18°C . Fresh fillets were analysed for moisture, fat, ash and protein according to the methods of AOAC (1960).

The changes in appearance, desiccation, discolouration and loss in weight (Table 2) of the frozen material were noted at specific intervals. The frozen fillets were thawed at room temperature and the yield determined (Table 3). The peroxide value (PV) and free fatty acid (FFA) contents of the fillets after thawing were estimated by the methods of Lea (1952) and AOCS (1946) respectively. The organoleptic tests were carried out after cooking the thawed fillets in 2% sodium chloride solution for 15 minutes.

* The samples have been designated by these numbers in the tables

Results and Discussion

The weight of individual cat fish used for the study varied from 1.6 to 2 kg and the yields of skinless fillets from 25-28% of the weight of whole fish. Weights of individual fillets ranged from 200 to 250 g. The fillets after washing in water had no unpleasant odour and their appearance was quite attractive. The proximate composition of the fillets is given in Table 1.

Maximum loss in weight occurred in individually frozen fillets stored without

Table 1. *Proximate composition of cat fish fillets*

Moisture %	77.560
Protein % (TN x 6.25)	17.470
Fat %	3.212
Ash %	0.816

Table 2. *Weight loss during storage of frozen fillets at -18°C (as % in frozen condition)*

Storage period weeks	Frozen fillet samples				
	1	2	3	4	5
5	0.68	0.33	0.25	Nil	Nil
10	2.73	0.33	1.05	Nil	Nil
16	6.14	0.33	1.37	Nil	0.32
20	—	—	1.37	Nil	0.32
23	—	0.49	1.62	0.25	0.32
27	—	0.58	—	0.50	0.64
32	—	—	—	—	0.64

Table 3. *Percentage yield of thawed fillets*

Storage period weeks	Frozen fillet samples				
	1	2	3	4	5
0	94.70	95.65	94.86	95.53	95.40
5	93.33	95.33	93.75	95.00	95.50
10	89.67	94.67	92.50	94.00	95.00
16	83.10	92.00	90.00	93.20	94.00
20	—	91.06	90.46	—	—
23	—	89.00	88.33	93.75	94.25
27	—	86.67	—	88.25	89.80
32	—	—	—	—	88.00

any packaging. But in the case of individual fillets frozen after wrapping in 200 gauge polythene sheet and stored at -18°C the loss due to evaporation of moisture from the surfaces was reduced considerably. Fillets packed in waxed cartons without any lining showed a loss of only 1.62% in weight after 23 weeks. Very little loss of weight occurred when the fillets were packed as blocks without adding glaze water in waxed cartons with an inside polythene lining. In the case of fillets frozen as glazed blocks and packed in polythene lined waxed cartons, the slight decrease in weight was due to the evaporation of glaze covering the frozen fillets and there was no loss of material due to desiccation as the frozen fillets were well protected by glaze as well as by packaging.

The yield of thawed fillets after 16 weeks was minimum (83.1%) in individually frozen fillets without any wrapping (Table 3). This was owing to the loss in weight during storage due to desiccation from the exposed surface of the fillets and to the drip loss occurring during thawing. Maximum yields of thawed fillets were obtained from samples packed in waxed cartons lined inside with polythene, as there was no loss due to desiccation during frozen storage. In these cases, the yields of the fillets after thawing were influenced by the quantities of drip lost during thawing. The drip losses (weight loss during thawing) in these samples slowly increased during storage.

The physical appearance of the frozen fillets and the organoleptic qualities of the

Table 4. Changes in physical appearance of frozen fillets during storage and organoleptic quality of the thawed fillets (after cooking)

Storage period weeks	Frozen fillet samples					Thawed fillets after cooking				
	1	2	3	4	5	1	2	3	4	5
0	Good	Good	Good	Good	Good, completely covered with water glaze	Good	Good	Good	Good	Good
5	Slight desiccation and slight yellow discoloration	Good	Good	Good	-do-	Fair	Good	Good	Good	Good
10	Through-out desiccation and yellow discoloration	Good	Fair to good	Good	-do-	Poor, spongy texture and rancid flavour	Good	Fair, slight spongy texture	Good	Good
16	—	Ice formation on the surface at certain places	Slight yellow discoloration	Good	-do-	—	Fair to good	Fair to poor, slight off odour	Fair to good	Good
20	—	Slight discoloration	—	Ice crystal formation at few places on the surface	-do-	—	Fair	Poor, off odour and flavour	Fair	Fair to good
23	—	-do-	—	-do-	-do-	—	Fair to poor	—	Fair	Fair
27	—	-do-	—	-do-	-do-	—	Poor, off odour and flavour	—	Fair to poor	Fair
32	—	—	—	-do-	-do-	—	—	—	Poor	Fair to poor

Table 5. *Changes in PV and FFA of frozen cat fish fillets during storage at—18°C*

Sample	Storage period weeks	FFA (as oleic acid %)	PV (ml N/500 thio/g fat)
1	0	1.82	2.68
	5	3.15	12.04
	10	3.52	28.61
2	0	2.06	2.43
	20	4.60	16.12
3	0	2.13	2.18
	10	3.94	21.05
4	0	1.88	1.18
	20	4.01	20.06
	23	3.87	32.16
5	0	1.73	2.52
	23	3.12	21.74
	27	4.86	27.61

thawed and cooked fillets were significantly influenced by packaging (Table 4). The individually frozen fillets stored without any packaging developed desiccation and discolouration after 5 weeks at—18°C. After 10 weeks, these samples were completely discoloured and desiccation occurred on the entire surfaces of the frozen fillets. The samples also exhibited high rancidity. In block frozen samples without water glaze and packed in waxed cartons, the texture of the fillets became slightly spongy after 10 weeks. After 16 weeks, the samples became unacceptable with the development

of rancid flavour, off odours, desiccation and discolouration. This is also accompanied by the increase in the peroxide values and free fatty acids (Table 5). The individually frozen cat fish fillets packed in 200 gauge polythene paper had a shelf life of 20 weeks at—18°C. Maximum storage life was shown by fillets frozen as glazed block and stored after packing in polythene lined waxed cartons.

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Bacteriology of Spoilage of Canned Prawns

V. NARAYANAN NAMBIAR*

Central Institute of Fisheries Technology, Cochin-682 029

Spoilage characteristics of different types of bacteria isolated from bacteriologically defective cans and processing factory environs were studied by inoculating pure cultures into sterile prawn meat. The pattern of spoilage, namely, production of off odour, bulging of the cans and disintegration of meat were observed. Data on spoilage under aerobic as well as anaerobic conditions are presented. Most of the cultures produced some kind of spoilage, though differences were observed in the extent of spoilage produced by different types of bacteria. Gram positive spore formers were found to be the major spoilers and the extent of spoilage was more with mixed cultures.

Canned prawns undergo spoilage due to bacterial action and as per the present standards they should be examined for commercial sterility before being exported. Commercial sterility may be defined as that condition in which all *Clostridium botulinum* spores and all other pathogenic bacteria, as well as more heat resistant organisms, which if present, could produce spoilage under normal conditions of storage and distribution have been destroyed (Denny, 1970). Evancho *et al.* (1973) have specified the conditions necessary for sterility testing of heat processed canned foods and Denny (1970, 1972) described the methods for the determination of commercial sterility of low-acid canned foods. Chaudhuri *et al.* (1970) have studied the factors controlling sterility in canned prawns and stressed the necessity of strict hygienic conditions in the processing centres for reducing the bacterial spoilage of canned prawns. Bacterial spoilage of canned foods may occur due to under processing or post-process reinfection due to defects in seam and rough handling. Many cases of food poisoning are due to post-process contamination. Nambiar & Iyer (1970) have made a detailed study of the type of micro-organisms present in bacteriologically defective canned prawns. As is the case with spoilage of fish (Adams *et al.*, 1964), it is very unlikely that all the bacteria are equally active in spoilage.

Though the bacteriology of spoilage of fish muscle had been studied extensively (Shewan *et al.*, 1960; Lerke *et al.*, 1963; Adams *et al.*, 1964; Herbert *et al.*, 1971), very little information is available on the bacteriology of spoilage in canned prawns. Farber & Ferro (1956) have made a report on the volatile reducing substances and volatile nitrogen compounds in relation to spoilage in canned fish and Tanikawa *et al.* (1967) have studied the bacterial action involved in can swelling and blackening of canned baby clams. Put *et al.* (1972) have reviewed the mechanism of microbial leaker spoilage of canned foods. Mc Meekin (1975, 1977) has studied the ability of pure cultures of bacteria to produce strong off odours in chicken breast muscle and chicken leg muscles. Much work has not been done on the ability of pure cultures to produce spoilage and off odours in canned prawns. The present study was undertaken to collect information on the ability of pure cultures of bacteria isolated from bacteriologically defective cans and processing factory environs to produce spoilage of canned prawns.

Materials and Methods

Fresh prawns (*Metapenaeus dobsoni*) of uniform size were obtained from the landing places in and around Cochin. The material was cleaned, washed, peeled and deveined manually. The deveined meat was washed thoroughly in potable water and blanched in 7% brine containing 0.2% citric acid for four minutes (Varma *et al.*,

*Present address: Bombay Research Centre of Central Institute of Fisheries Technology, Bombay-400 005

1969). After draining off the brine, the blanched meat was immediately cooled under a fan and packed. For spoilage studies under aerobic conditions 50 g of the blanched material was packed in 150 ml conical flasks with 50 ml of 3% brine containing 0.2% citric acid, plugged with cotton, sterilized at 115.2°C for 18 min, cooled and used for further studies. For spoilage studies under anaerobic conditions good quality cans of 301 x 206 were used. The cans were washed properly. 128 g of blanched meat was packed with 90 ml of 3% brine containing 0.2% citric acid. After steam exhausting for 10 min, the cans were double seamed and sterilized at 115.2°C for 18 min. Immediately after autoclaving the cans were taken out, cooled in chlorinated cooling water (5 ppm available chlorine), wiped dry and used for further studies.

Pure cultures of bacteria isolated from bacteriologically defective cans, as well as processing factory environs were used for this study (Nambiar & Iyer 1970, 1971, 1973). Representative cultures having differences in one or more biochemical characteristics were selected from the isolates. The cultures, other than *Clostridium* sp. were maintained on nutrient agar slants and subcultured and tested for purity every fortnight. Suspensions of these cultures were prepared by harvesting 48-72 h nutrient agar slant cultures using normal saline. The suspensions were diluted with normal saline to give a count of approximately 10^6 cells per ml. In the case of *Clostridium* the cultures were maintained in cooked meat medium. For use, stock cultures were inoculated into thioglycollate broth and the cells from 48-72 h cultures were separated by centrifuging at 3000 rpm for 5 min at room temperature ($28 \pm 1^\circ\text{C}$), washed and suspended in normal saline to give a count of approximately 10^6 cells per ml. In addition to the 200 pure cultures, 120 mixed cultures prepared by mixing two cultures were also used in the study. Fifteen strains of *Bacillus* sp., four gram negative rods, two gram positive cocci and two gram negative cocci were used in the preparation of mixed cultures. They were mixed in the order of one *Bacillus* with one gram negative rod or one *Bacillus* with a *Cocci*. Equal volumes of suspensions of

the individual cultures containing 5×10^6 cells per ml were mixed to give a final count of 1×10^6 cells per ml.

In the case of prawns packed in conical flasks, one ml each of the bacterial suspension (other than *Clostridium*) was inoculated into two flasks using a sterile graduated pipette, a strip of lead acetate paper was inserted into each flask, without touching the contents, under aseptic conditions plugged with cotton and stored at room temperature ($28 \pm 1^\circ\text{C}$) for two weeks. The contents were examined for changes in odour, colour, texture, pH and the lead acetate paper was observed for blackening, if any. The contents were shaken to observe disintegration.

The cans packed with prawns were inoculated with the bacterial cultures by piercing a hole in the lid of the can using a sterile sharp needle. One ml each of the suspension was inoculated into a set of two cans using a sterile syringe. The hole was closed by soldering. The cans were incubated at room temperature for 2 weeks and observed for bulging, if any, opened and the contents examined. Odour, texture, colour, pH and disintegration of the contents were noted. The bacteria from the cans were examined for their morphology.

In another set of experiments, the prawns in conical flasks were inoculated with bacterial suspensions and steamed for 5 min and further incubated at room temperature as above, to study whether the bacteria could survive steaming for 5 min. Suspensions of bacterial strains were inoculated into the cans just before retorting and the cans were incubated and observed as before, to study the effect of autoclaving on the bacterial strains.

The effect of prolonged incubation of the cans on the survival, growth and spoilage characteristics of the different types of bacteria was also studied by inoculating the bacterial suspensions into the cans and incubating the cans for 1 year. A few cans were examined every month for spoilage and changes in the morphology of the bacteria.

Results and Discussion

The total number of bacterial strains and their types used in this study are shown in Table 1. The spoilage pattern of all the cultures except the *Clostridium* under aerobic conditions is presented in Table 2. The pattern of spoilage like production of off odour, stale odour, hydrogen sulphide, pH changes and disintegration of the meat are presented in terms of percentages of each type of bacteria showing such changes. Disintegration of meat was observed in all cases except by a few cocci, gram negative rods and mixed cultures. It was comparatively high with gram positive spore formers and with mixtures of spore formers with gram negative rods. Although all the spore formers which were components of the mixed cultures produced hydrogen sulphide when used alone, only six of them did so when mixed with gram negative rods and none produced hydrogen

sulphide when mixed with gram positive and negative cocci. Similarly, all the spore formers which produced off odour when used alone did not do so when mixed with other cultures. Only eight spore formers when mixed with a gram negative rod and two spore formers when mixed with a gram negative cocci produced off odour. It may be observed from the results that none of the gram negative rods, gram positive and negative cocci produced hydrogen sulphide or off odour when used alone. In the case of five spore formers when mixed with the two gram positive cocci, no change was observed. These changes in the pattern of spoilage with mixed cultures may be either due to the inhibition of the particular reaction or due to the competitive overgrowth of the less reactive culture.

Table 1. Microorganisms tested for spoilage characteristics

Type of bacteria	No. of strains
Gram positive spore formers	
(a) <i>Bacillus</i> sp.	80
(b) <i>Clostridium</i> sp.	34
Gram positive non-spore forming rods	24
Gram positive cocci	24
Gram negative cocci	12
Gram negative rods	26
Total	200

Table 3 presents the spoilage pattern under anaerobic conditions. Among the gram positive spore formers all the *Clostridium* produced off odour and bulging of the cans whereas no bulging was observed in the case of *Bacillus* and only 5% of them produced off odour, the rest producing stale odour. Although none of the cultures other than the *Clostridium* produced bulging of the cans when used alone, six strains of the *Bacillus* produced bulging when used along with a gram negative rod. Similarly, whereas only four *Bacillus* produced off odour when used alone, eight of them produced off odour when mixed with two gram negative rods. This may be due to the combined effect of the components of the mixed cultures under anaerobic condi-

Table 2. Spoilage pattern under aerobic conditions*

Type of bacteria	No. of strains	Stale odour	Percentage showing		pH change	Disintegration
			Off odour	H ₂ S production		
<i>Bacillus</i> sp.	80	75.0	25.0	90.0	25.0	100.00
Gram positive non-spore forming rods	24	100.0	Nil	50.0	16.5	100.00
Gram positive cocci	24	80.0	Nil	Nil	Nil	80.0
Gram negative rods	26	75.0	Nil	Nil	Nil	75.0
Gram negative cocci	12	85.0	Nil	Nil	Nil	85.0
Mixed cultures	120	85.0	8.0	20.0	25.0	93.0

*Bacterial cultures inoculated into prawn meat packed in conical flasks

Table 3. Spoilage pattern under anaerobic conditions*

Type of bacteria	No. of strains	Stale odour	Percentage showing		Disintegration
			Off odour	Bulging	
<i>Bacillus</i> sp.	80	95.0	5.0	Nil	100.0
<i>Clostridium</i> sp.	34	Nil	100.0	100.0	100.0
Gram positive non-spore forming rods	24	66.6	Nil	Nil	50.0
Gram positive cocci	24	50.0	Nil	Nil	50.0
Gram negative rods	26	100.0	Nil	Nil	100.0
Gram negative cocci	12	75.0	Nil	Nil	75.0
Mixed cultures	120	80.0	13.0	5.0	98.0

*Bacterial cultures inoculated into prawns packed in cans

tions. In the case of cans which showed bulging, the colour of the brine changed black and the meat became pale. Small changes in pH were also observed in bulged cans whereas in all other cases the pH was 6. Though disintegration was produced by all the *Bacillus*, *Clostridium*, gram negative rods and 98% of the mixed cultures, it was 75% in the case of gram negative cocci and only 50% in the case of gram positive non-spore forming rods and gram positive cocci. Similarly, changes in odour was observed with 66.6% of gram positive non-spore forming rods, 50% of gram positive cocci and 75% of gram negative cocci, whereas it was almost 100% with other cultures. Disintegration of the meat was very high with *Clostridium*, moderate with *Bacillus* and mixed cultures and comparatively less with other cultures. Though a few cultures did not produce any change in the contents, all cultures survived in the cans as the respective strains could be isolated from the cans.

Results of the experiments carried out to study the effect of steaming after inoculation of bacteria into prawn meat showed that except 60% of the *Bacillus* and 50% of the mixed cultures, others did not produce spoilage as they were all destroyed during steaming. Even in the case of cultures which showed growth, a delay was observed in the onset of spoilage. Production of stale odour and disintegration of meat were the changes observed in these cases.

None of the cultures survived sterilization and no changes were observed in

the can contents when the cultures were inoculated into the cans before sterilization. Prolonged incubation of the cans after inoculation did not show any change in the pattern of spoilage. The cultures were found to survive such incubation without any change in their morphology.

Although canning of prawns involves heat treatment which is sufficient to destroy enzymes, micro-organisms and their toxins, canned prawns nevertheless undergo microbial spoilage under certain conditions. The canning operation may fail in two respects. First, if the cans are not subjected to sufficient heat to effectively sterilize the contents, the product may spoil on storage. This type of spoilage is known as under processing and the factors contributing to this are (a) faulty retort operation, (b) exceptional heat resistance of some bacteria present in the material, (c) excessive microbial contamination of the raw material, where the heat treatment may not be sufficient to reduce the contamination to commercial sterility level and (d) inefficient cooling of the cans after retorting which may favour the growth of thermophilic organisms surviving the heat treatment. In all these cases, the bacteriological analysis of the cans may usually show a single type of organism, especially of the spore forming type, except in cases of gross under-processing where a mixed culture may be observed. The second type of spoilage is due to post process reinfection wherein micro-organisms from the surrounding environment may gain access to the contents through leaks in the container. This type of spoilage is called

leaker spoilage and is the most significant type of spoilage in canned foods. The major factor contributing to this type of spoilage is seam defects either in the construction of the can or due to straining of the seams during processing, owing to the sudden fluctuations in the pressure outside the cans during sterilization and cooling, or during rough handling. Water used for cooling the cans after retorting is the major source of contamination due to leakage. As the cooling water is often chlorinated, bacteria other than the spore formers may be absent and hence in this type of spoilage also a single type of bacteria of the spore forming type may be observed, except in cases of excessive contamination of cooling water and rough handling of the cans. Nambiar & Iyer (1970, 1971) have observed that gram positive spore formers are predominant in bacteriologically defective cans though other types are observed occasionally. Though in the spoilage of fish muscle gram negative organisms of the *Pseudomonas* and *Achromobacter* are the major spoilers as reported by Adams *et al.* (1964), the results of the present investigation show that gram positive spore formers are the major spoilers in canned prawns. It is also observed that other types of organisms produce spoilage in canned prawns, though to a lesser degree. Majority of the bacterial cultures produce changes in the odour and texture of the material though no visible change in the external appearance of the cans is produced. The difference observed in the extent of spoilage caused by organisms other than gram positive spore formers under anaerobic conditions may be due to the unfavourable condition for the growth and proliferation of these organisms. It is also observed that with mixed cultures the extent of spoilage is more when compared to that of pure cultures.

The present study shows that bacteria, if present in sufficient numbers, irrespective of their type, can cause spoilage of canned prawns and hence the bacteriological examination of canned prawns for commercial sterility is all the more important. To prevent bacterial spoilage of canned prawns, it is necessary to ensure that the cans are given adequate heat treatment and good quality water is used for cooling.

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Transportation of Fish - iii Biochemical Changes in Fish During Transportation in Insulated Containers

P. R. G. VARMA*, A. P. VALSAN** and P. V. PRABHU*

Veraval Research Centre of Central Institute of Fisheries Technology, Veraval-362 265

The changes in the biochemistry of different varieties of iced fish during transportation in thermocole lined second hand teachests from Veraval to Bombay and Delhi are discussed. The moisture increased for all the varieties except for eel and hilsa at Bombay. TMAN and TVN increased in all cases. Maximum increase of TMAN and TVN was observed in seer fish.

In a large tropical country like India, where some of the potential fish consuming centres are thousands of kilometres away from the major fish landing centres, an effective system of handling and transportation is very essential. An extensive survey conducted by the Central Institute of Fisheries Technology, Cochin at the major fish consuming centres like Bombay and Delhi had revealed large variations in the quality of fish arriving in these markets (Anon, 1973).

A good knowledge of the biochemical changes that are taking place during transportation will help to develop suitable handling and transportation methods. The biochemical changes taking place in the case of frozen fish packed in insulated containers and stored at different temperatures have been studied previously (Perigreen, 1968), but very little is known about the biochemical changes taking place during transportation of iced fish. So this study was taken up under the "All India Co-ordinated Research Project on Transportation of Fresh Fish."

Materials and Methods

The container used was thermocole lined second hand tea-chests (Venkataraman *et al.*, 1976). Fish to ice ratio 1:1 was used. After packing, the boxes were des-

patched either to Bombay or Delhi in non-insulated rail wagons. The fishes used for the study included hilsa (*Hilsa ilisha* and *Hilsa toli*), silver pomfret (*Pampus argenteus*), black pomfret (*Parastromateus niger*), dhoma (*Otolithes sp.*), seer fish (*Scomberomorus guttatus*), eel (*Muroenesox cinereus*) and dai (*Chirocentrus dorab*). The biochemical analyses were carried out before despatch from Veraval and immediately after receipt at Bombay and Delhi. Moisture was estimated by the method of AOAC (1960) and trimethyl amino nitrogen (TMAN) and total volatile nitrogen (TVN) from the trichloroacetic acid extract by the method of Conway (1947).

Results and Discussion

Table 1 shows the average changes in the percentage of moisture in different varieties of fishes during transportation from Veraval to Bombay and Delhi. The percentage of moisture has gone up in almost all the varieties of fishes except in eel and hilsa despatched to Bombay. The average decrease in eel and hilsa was 0.65 and 1.11 respectively. But the percentages of moisture showed an increase for both these varieties at Delhi. This can be due to the prolonged contact with ice as the consignment took about 40 h to reach Delhi compared to 24 h to Bombay. At Bombay the minimum and maximum increase in the moisture were in cat fish and dai respectively while in Delhi minimum was for dhoma and maximum for pomfret. Both at Bombay

Present address: *Central Institute of Fisheries Technology, Cochin-682 029

**Bombay Research Centre of Central Institute of Fisheries Technology, Bombay-400 005

and Delhi the TMAN showed increase during transportation, but it was more pronounced at Delhi (Table 2). This is also due to the longer time required for transportation. The highest increase of TMAN was in seer fish and the lowest in silver pomfret and hilsa. In all the cases the TVN registered an increase, the increase being more during transportation to Delhi (Table 3). As in TMAN, the highest increase in TVN was noticed in seer fish. This may be due to the soft muscle of the seer fish which is susceptible to quick spoilage.

Table 1. *Average percentage changes in moisture for different varieties of fish during transportation from Veraval to Bombay and Delhi*

Fish	Bombay	Delhi
Eel	— 0.65	+ 2.230
Silver pomfret	+ 1.14	+ 4.365
Black pomfret	+ 1.73	—
Dhoma	+ 1.39	+ 1.000
Dai	+ 1.97	+ 1.120
Hilsa	— 1.11	+ 2.934
Cat fish	+ 0.15	—
Seer	+ 0.42	+ 2.320
Ribbon fish	—	+ 2.200
<i>Lactarius</i>	—	+ 1.810
Prawn	—	+ 3.890

Table 2. *Average values of TMAN (mg/100g)*

Fish	Transportation to Bombay		Transportation to Delhi	
	Before despatch	After arrival	Before despatch	After arrival
Hilsa	1.420	1.559	1.510	2.302
Eel	1.585	1.618	2.028	2.586
Silver pomfret	0.825	1.004	0.680	2.331
Black pomfret	1.243	1.280	1.615	2.651
Dhoma	1.245	1.201	1.226	2.610
Perch	0.858	1.028	0.887	2.364
Seer	0.895	1.619	0.602	2.725
Dai	0.852	1.010	0.508	2.352
Cat fish	0.580	0.725	—	—

Table 3. *Average values of TVN (mg/100g)*

Fish	Transportation to Bombay		Transportation to Delhi	
	Before despatch	After arrival	Before despatch	After arrival
Hilsa	9.441	14.97	10.050	15.86
Eel	10.160	13.36	7.882	13.03
Silver pomfret	8.616	14.37	6.668	17.69
Black pomfret	9.550	15.26	11.460	14.02
Dhoma	12.420	14.34	8.570	17.84
Perch	9.943	15.12	11.990	15.01
Seer	12.130	21.96	10.110	27.57
Dai	9.302	13.13	8.845	17.50
Cat fish	11.040	14.94	—	—

Thanks are due to Dr. R. Raghu Prasad, Assistant Director General, ICAR for his guidance and interest in this project study, to Shri R. Venkataraman for encouragement and to Shri G. K. Kuriyan, Director, Central Institute of Fisheries Technology for permission to publish the paper.

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Purification and Properties of Aspartate Aminotransferase (E.C. 2.6.1.1) from Skeletal Muscle of *Cirrhina mrigala*

S. K. CHHATBAR* and N. K. VELANKAR

Central Institute of Fisheries Education, Bombay-400 061

Aspartate aminotransferase (E. C. 2.6.1.1.) from the skeletal muscle of fresh water fish *Cirrhina mrigala* has been purified 40 fold by ammonium sulphate fractionation, adsorption on alumina C_8 gel and chromatography using DEAE-cellulose column and the properties of the purified enzyme studied. The pH optimum of the enzyme is 7.8. The K_m value of aspartic acid and 2-oxoglutaric acid are found to be 2.8×10^{-3} M and 1.0×10^{-4} M respectively. The activity of enzyme is inhibited by p-chloromercuribenzoate, hydroxylamine hydrochloride and sodium cyanide. The inhibition by p-chloromercuribenzoate is reversed by reduced glutathione, B-mercaptoethanol and cysteine. Dicarboxylic acids such as maleic acid, malic acid and succinic acid inhibit the enzyme activity. The enzyme is not activated by any of the metal ions tested and heavy metal ions such as mercury and silver strongly inhibit the enzyme activity.

The tissues of all species of animals contain enzymes which catalyze the process of biological transamination. Transamination reactions represent a prime mechanism for the synthesis and deamination of amino acids in the tissues. The aminotransferases found in the tissues are mainly aspartate aminotransferase, AAT (1-aspartate: 2-oxoglutarate aminotransferase E. C. 2.6.1.1) and alanine aminotransferase, ALAT (1-alanine: 2-oxoglutarate aminotransferase E. C. 2.6.1.2) (Meister, 1962). The purification and properties of aminotransferases from various animal and plant sources and from micro-organisms have been reported by several workers as reported by Chhatbar (1977). Chhatbar (1977) observed that AAT activity was present in sarcoplasm as well in mitochondria of skeletal muscle of fish. Both the isoenzymes had different electrophoretic mobilities and could be separated by electrophoresis.

In this paper purification and properties of sarcoplasmic aspartate aminotransferase from white skeletal muscle of fresh-water fish, mrigal is reported.

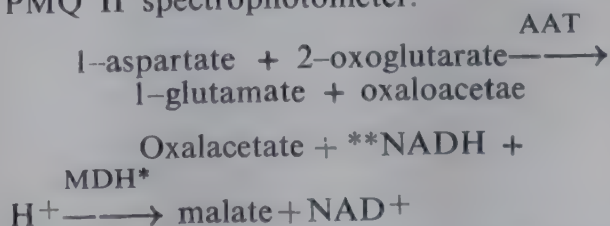
Materials and Methods

The chemicals used were of analytical grade. White skeletal muscle of mrigal immediately killed was the source of the

enzyme. All purification experiments were performed at 0 to 5°C. Unless otherwise specified, the buffer used throughout was 0.1 M phosphate buffer, pH 7.6 and the centrifugation was carried out at 10,000 g for 10 min in a refrigerated IEC centrifuge. Percent ammonium sulphate used for fractionation was based on Green & Hughes (1955), though the solutions were maintained at 0 to 5°C. At every stage of purification, the preparation was assayed for protein content and subjected to polyacrylamide disc-gel electrophoresis. Alanine aminotransferase activity was determined (Bergmeyer & Bernt, 1965) at each stage of purification.

Assay of AAT activity

AAT activity was determined by the coupled enzyme reaction according to Bergmeyer & Bernt (1965) using Carl-Zeiss PMQ II spectrophotometer.



The oxidation of NADH is measured by decrease in absorbancy at 340 nm. One unit

MDH* malate dehydrogenase

NAD⁺ nicotinamide adenine dinucleotide

NADH** reduced NAD

*Present address: Export Inspection Agency, Aman Chambers, 113, M. Karve Road, Bombay-400 004

of enzyme activity is defined as that activity which catalyses the oxidation of one micromole of substrate per min at 30°C.

Protein estimation

The protein content in the enzyme preparations were determined with Folin-Ciocalteu reagent according to Lowry *et al.* (1951) using crystalline bovine serum albumin (Sigma) as the standard. Protein content in various fractions obtained by DEAE-cellulose chromatography was expressed as absorbance at 280 nm.

Electrophoresis

Disc electrophoresis in polyacrylamide gel (7.5%) was performed as described by Smith (1960). Electrophoresis on cellulose acetate membrane (CAM) was performed (Smith, 1960) using Beckman model R-101 microzone electrophoresis cell (Beckman Instruments Inc., California, Manual RM-IM-3, 1965).

Purification of aspartate aminotransferase

About 50 g of skeletal muscle was ground with minimum amount of phosphate buffer using a glass mortar and pestle. The partially homogenized tissue was further homogenized using Potter-Elvehjem type of homogenizer operating at variable speed. The homogenate (about 300 ml) was stirred for 2-3 h followed by centrifugation at 500 g for 10 min to separate the large cellular debris. The supernatant was further centrifuged at 20,000 g for 10 min.

First ammonium sulphate fractionation

Solid ammonium sulphate (special for enzyme work, Sarabhai Chemicals) was gradually added to the extract (300 ml) with continuous stirring to 30% saturation. The pH was maintained constant by addition of ammonia solution. The saturated homogenate was stirred for 2 h and centrifuged. The negligible amount of precipitate obtained was discarded. The supernatant was brought to 70% ammonium sulphate saturation and allowed to stand overnight in the cold. The precipitate was separated by centrifugation and dissolved

in phosphate buffer. It was dialysed against phosphate buffer containing 10^{-3} g/l pyridoxal phosphate (PLP) till it was free from ammonium sulphate.

Alumina C_8 treatment

Alumina C_8 gel was prepared as described by Dawson *et al.* (1969). The dialysate obtained was clarified by centrifugation. It was treated with alumina gel (0.2 mg gel/mg protein) and allowed to stand for 30 min. The mixture was centrifuged and the sedimented gel was discarded. The supernatant was further treated four times with fresh gel.

Second ammonium sulphate fractionation

The partially purified preparation was saturated to 70% with ammonium sulphate. The precipitate was separated by centrifugation.

DEAE cellulose chromatography

DEAE — cellulose (Whatman) was subjected to a standard cycle of washes with 0.1 N sodium hydroxide, 0.5 N hydrochloric acid and water as described by Himmelhoch (1971). DEAE-cellulose suspended in 0.01 M phosphate buffer, pH 7.5 containing 0.1 M sodium chloride and 10^{-3} g/l PLP was packed in a column (1 x 30 cm) and washed with 0.01 M phosphate buffer containing 0.1 M sodium chloride.

The precipitate obtained in the previous step was dissolved in 50 ml of 0.01 M buffer containing 0.1 M sodium chloride. It was dialysed against the latter supplemented with PLP, till free from ammonium sulphate. 15 ml of the dialysate was applied to the column and eluted with 0.01 M buffer containing 0.1 M sodium chloride at the rate of 5 ml per 30 min. 5 ml eluate was collected in each tube. The concentration of sodium chloride in elution buffer was increased to 0.2 M and 0.3 M respectively. The contents of six tubes (15 to 20) were pooled together and dialysed against distilled water containing PLP to remove salts. The dialysate was saturated to 75% with ammonium sulphate and was stored in cold.

The characteristics of the purified enzyme such as requirement for co-factors, ultra-violet absorption spectrum and electrophoretic mobilities were studied. The enzyme kinetics and the influence of pH, inhibitors, dicarboxylic acids and metal ions on enzyme activity were studied. The standard 3 ml reaction mixture contained 0.1 M phosphate buffer pH 7.6, 2.5×10^{-1} M L-aspartate, 0.2 M 2-oxoglutarate, 1.2×10^{-2} M NADH, 25 mg MDH and 87 mg of purified enzyme protein. 1 ml of buffer in reaction mixture was replaced with equal volume of inhibitor, dicarboxylic acid or metal ion solution as the case may be.

The influence of aspartic acid concentration on AAT activity was studied by varying its final concentration from 0.0005 M to 0.04 M. The concentration of co-substrate, 2-oxoglutaric acid was kept constant at 0.2 M. The influence of 2-oxoglutaric acid on enzyme activity was determined by varying its concentration between 0.001 M and 0.1 M. The Michaelis constants (apparent K_m values) for aspartic acid and 2-oxoglutaric acid were obtained from Lineweaver-Burk plots.

The influence of pH on purified AAT was studied under assay conditions using 0.1 M phosphate buffer and 0.1 M Tris-HCl buffer. An attempt was made to compare the pH behaviour of sarcoplasmic and mitochondrial AAT enzymes. Mitochondria from skeletal muscle of mrigal were isolated by differential centrifugation (Chhatbar, 1977). The mitochondria were

subjected to repeated freezing and thawing followed by incubation with 0.2 ml of 10% Triton x 100 at 37°C for 30 min. This was used as a source of mitochondrial AAT to study the influence of pH on its activity.

Results and Discussion

Table 1 reports the summary of the purification procedure. It is evident that AAT was purified about 40 fold by the methods adopted. The activity precipitated between 30–70% saturation with ammonium sulphate. First fractionation showed about 2.3 fold increase in the activity. At this stage, the fraction also possessed significant level of ALAT activity. Though, the second fractionation did not accomplish any significant concentration in AAT activity, it was useful in concentrating the protein by precipitation. Treatment with alumina gel was found useful for attaining 7 fold purification. Chromatography on DEAE—cellulose achieved maximum purification of AAT. The elution profile of AAT is shown in Fig.1. It indicated a very sharp peak of unadsorbed protein, devoid of AAT activity. It was found that AAT was eluted with 0.2M sodium chloride concentration.

The purified enzyme was relatively stable at lower temperatures of storage. It migrated as a single band of protein in polyacrylamide gel electrophoresis (Fig.2). It was devoid of ALAT activity, though the latter was detected during the first stage of purification. This indicates the

Table 1. Purification of aspartate aminotransferase E.C. 2.6.1.1 from skeletal muscle of *Cirrhina mrigala*

Step	Volume ml	Enzyme activity U/ml	Total enzyme activity	Protein mg/ml	Specific activity U/mg	Fold purification	Yield %
Homogenate	300	0.48	144.0	10.67	0.045	—	100
First ammonium sulphate fractionation	300	0.55	165.0	5.50	0.100	2.3	115
Alumina C_8 treatment	300	0.40	120.0	1.26	0.317	7.0	83
Second ammonium sulphate fractionation	50	1.80	90.0	5.29	0.340	7.6	63
DEAE-cellulose chromatography (pooled from 3 sets)	90	0.49	44.1	0.27	1.810	40.0	30

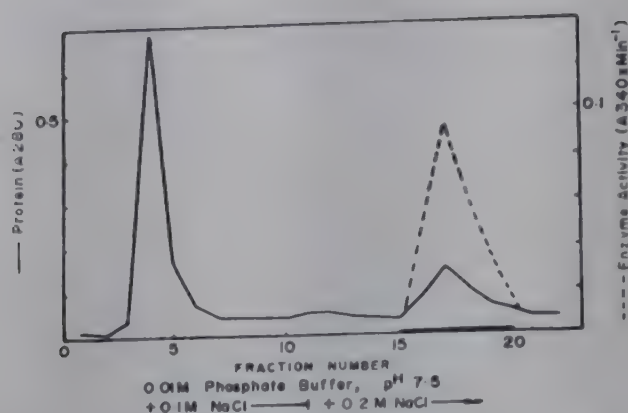


Fig. 1. DEAE cellulose chromatography of fish muscle aspartate aminotransferase

general observation that AAT and ALAT are two distinct enzymes. The purified AAT exhibited absorption peak at 276 nm in ultraviolet region (Fig. 3).

It was observed that the dialysis of the enzyme against phosphate buffer or distilled water resulted in loss of activity. The enzyme could be reactivated by PLP. Bell (1968) observed that AAT from liver of salmon was activated by PLP but not by pyridoxal, pyridoxamine or pyridoxine. In the present studies, it was found advantageous to incorporate PLP in dialysing buffer during purification stages.

Fig. 4 shows electrophoretic mobilities of AAT on CAM at different pH values. It is noted that the enzyme has an isoelectric point at about pH 5. Earlier, Marino *et al.* (1965) made similar observations with purified ox heart AAT.

Under the standard assay conditions, it was noted that the rate of the enzyme reaction was linear upto 50 min and the product formation was directly proportional to the enzyme concentration upto 29.1 μg protein.

The K_m value of purified AAT for aspartic acid was found to be $2.8 \times 10^{-3} \text{M}$ (Fig. 5). It was $1.0 \times 10^{-4} \text{M}$ for 2-oxoglutaric acid (Fig. 6). Fig. 7 shows that the plot of activity against pH as a bell-shaped curve with pH optimum at 7.8. It also shows pH behaviour of crude mitochondrial AAT and is distinctly different from that of purified sarcoplasmic AAT. It has broad



Fig. 2. Polyacrylamide gel electrophoresis of purified aspartate aminotransferase of fish skeletal muscle

pH optimum between 6.0 and 8.0. These observations confirm the presence of at least two distinctly separate sarcoplasmic and mitochondrial AAT proteins in fish skeletal muscle.

McAllan & Chefurka (1961) found that roach AAT had a marked pH optimum at 8.3. The pH optimum of human intestine AAT was found to be 7.6 using phosphate buffer (Ramaswamy & Radhakrishnan, 1964). Bell (1968) observed that partially purified AAT from salmon liver had no distinct optimum pH but the velocity of enzyme reaction was positively related to increasing pH over the range 5 to 10.

Table 2 presents the influence of inhibitors on AAT activity. It is seen that PCMB inhibits the activity even at low concentration (10^{-5}M). The inhibition by PCMB (10^{-4}M) is reversed by reduced glutathione, B-mercaptoethanol and cysteine (all 10^{-2}M), thus suggesting the presence of -SH, sulphhydryl groups in the protein molecule and their participation in enzyme activity. Katunuma *et al.* (1968) have observed that aminotransferases are

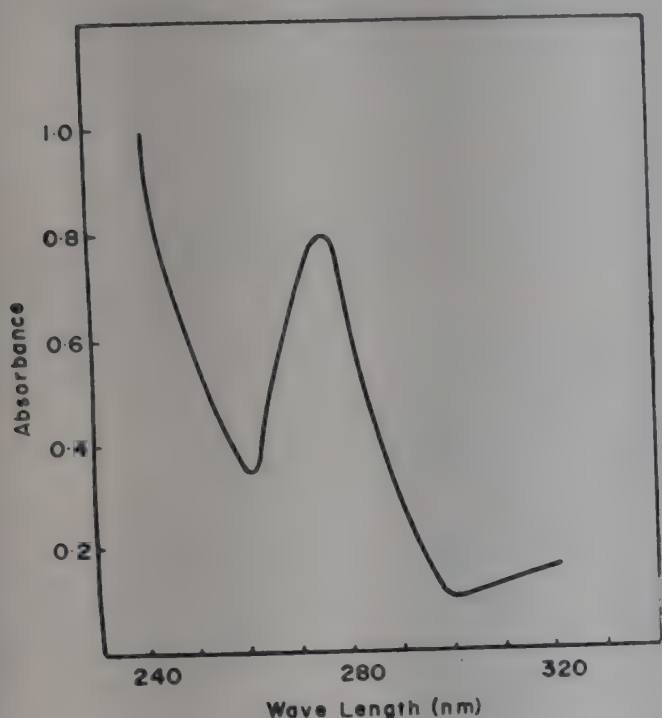


Fig. 3. Ultraviolet absorption spectrum of fish muscle aspartate aminotransferase (enzyme concentration 0.48 mg/ml)

Table 2. Influence of inhibitors on fish muscle aspartate aminotransferase

Inhibitor	Final concentration	*Activity % of control
p-chloromercury-benzoate (PCMB)	10^{-5}	91.00
	10^{-4}	47.00
	10^{-3}	5.80
PCMB (10^{-4})		
+ reduced glutathione	10^{-2}	91.00
+ cystein	10^{-2}	91.00
+ B-mercapto-ethanol	10^{-2}	100.00
Sodium cyanide	10^{-3}	77.00
Hydroxylamine hydrochloride	10^{-3}	5.80
Sodium arsenite	10^{-3}	100.00

* The activity in the control is taken as 100 in each experiment (standard assay conditions)

Table 3. *Influence of dicarboxylic acids on fish muscle aspartate aminotransferase

Compound	Inhibition %
Maleic acid	62
Malic acid	18
Succinic acid	10
Malonic acid	Nil
Oxalic acid	Nil

* The dicarboxylic acids were present in a final concentration of 4×10^{-2} M in standard assay mixture. The acids were neutralized with 1 N sodium hydroxide prior to addition

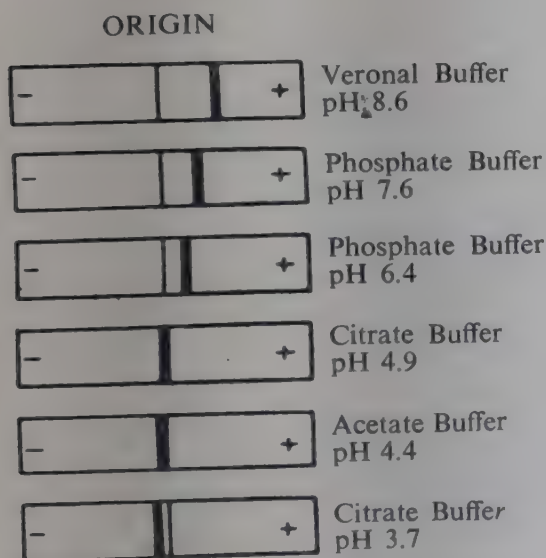


Fig. 4. Schematic representation of the electrophoretic mobility of fish muscle AAT at different pH values

sensitive to the reagents blocking sulphhydryl and carbonyl groups as well as to the antimetabolites of vitamin B-6.

Fish muscle AAT is inhibited by hydroxylamine hydrochloride at 10^{-3} M concentration whereas it is not substantially inhibited by sodium cyanide. It is also not affected by sodium arsenite.

The influence of dicarboxylic acids on AAT is presented in Table 3. It is observed that the enzyme is strongly inhibited by maleic acid. Malic acid and succinic acid are inhibitory to a certain extent whereas malonic and oxalic acids do not inhibit the enzyme activity. AAT is inhibited by several dicarboxylic acids probably due to the formation of an inactive

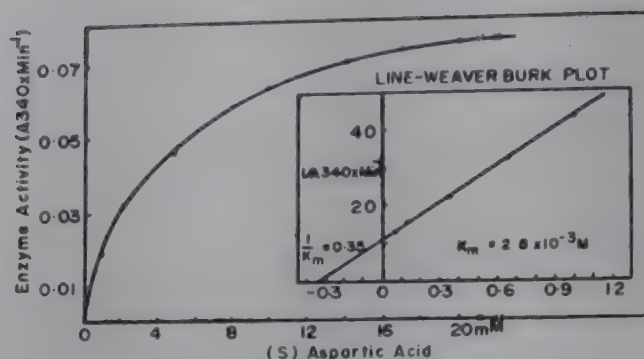


Fig. 5. Fish muscle aspartate aminotransferase: K_m value for aspartic acid

complex with the enzyme (Jenkins *et al.*, 1959). Michuda & Martinez (1970) found a correlation of the inhibition of transaminations with the affinity of the dicarboxylic acids to the pyridoxal form of the enzyme.

Table 4.* Influence of metal ions on fish muscle aspartate aminotransferase

Metal ion	Activity % of control
Mg^{+2}	100
Fe^{+2}	100
Pb^{+2}	20
Ag^{+2}	Nil
Fe^{+3}	97
Mn^{+2}	100
Hg^{+2}	2
Zn^{+2}	100
Cu^{+2}	97

* The final concentration of metal ions in the reaction mixture was 5×10^{-4} M under standard assay conditions. The sulphates of magnesium, ferrous, manganese, zinc and copper; chlorides of mercury and ferric; silver nitrate and lead acetate were used.

The results presented in Table 4 indicate that AAT was not activated by any of the metal ions tested, whereas heavy metal ions such as mercury and silver strongly inhibited the activity.

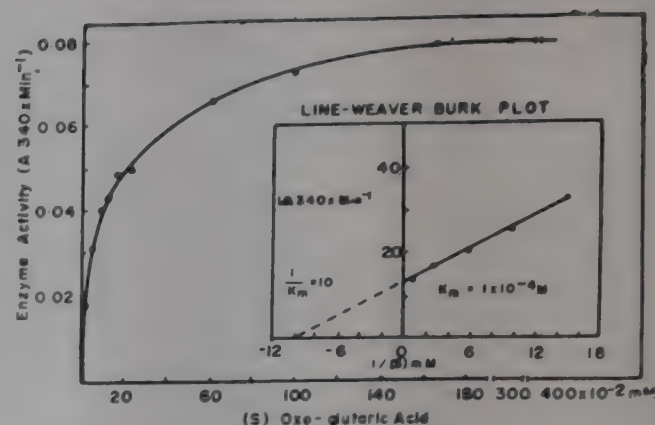


Fig. 6. Fish muscle aspartate aminotransferase: K_m value for oxo-glutaric acid

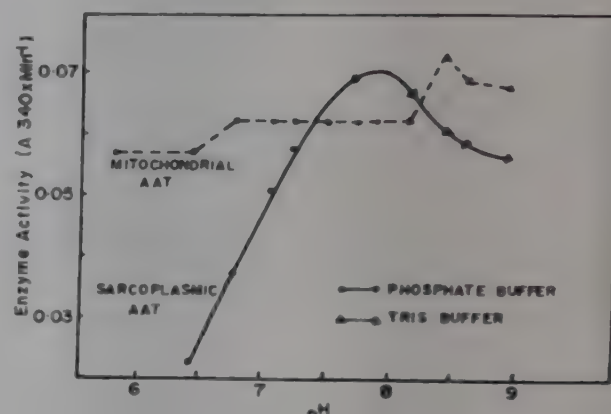


Fig. 7. Activity of fish muscle aspartate aminotransferase as a function of pH

The present investigations have shown that the properties and characteristics of AAT of the fish muscle are generally similar to those of AAT from other animal sources.

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Studies on Aminotransferase Enzymes in Fish and Shellfish

S. K. CHHATBAR* and N. K. VELANKAR

Central Institute of Fisheries Education, Bombay-400 061

The distribution of aspartate aminotransferase (AAT) and alanine aminotransferase (ALAT) activities in the skeletal muscle of several fish species is reported. AAT activity is found higher than ALAT activity and that the red muscle has higher aminotransferase activity than the white muscle. It is observed that 2-oxoglutaric acid has a wider scope as an amino group acceptor than pyruvic acid in the skeletal muscle of fish. The significance of transaminations in fish is discussed.

Aminotransferases are a group of enzymes that catalyse the process of biological transamination. The transamination reactions involve the transfer of the amino group of an amino acid to a keto acid with the formation from the latter of an amino acid and the generation of a new keto acid. These reactions represent a prime mechanism for the synthesis and deamination of various amino acids. The amino acids found in the tissues are mainly aspartate aminotransferase (AAT, 1-aspartate: 2-oxoglutarate aminotransferase E.C. 2.6.1.1) and alanine aminotransferase (ALAT, 1-alanine: 2-oxoglutarate aminotransferase, E.C. 2.6.1.2) (Braunstein, 1960; Meister, 1962).

AAT catalyses the reaction:

1-aspartate + 2-oxoglutarate $\rightleftharpoons$ 1-glutamate + oxaloacetate, whereas ALAT catalyses the reaction:
1-alanine + 2-oxoglutarate $\rightleftharpoons$ 1-glutamate + pyruvate

In fish; the transamination reactions probably play significant role at some stage in autolytic degradation of muscle proteins (Siebert & Schmitt, 1965). It is probable that the pool of free amino acids present in the fish muscle is utilized by transaminations to produce a variety of keto acids, some of these keto acids could be volatile and in addition some might be decarboxy-

lated and contribute a variety of aldehydes to the pool of carbonyl compounds which probably determine the overall flavour of fish (Murray & Burt, 1974). Bell (1968) discussed the practical value of AAT estimations in serum of salmon for the distinction of apparently healthy fish from those treated with the hepatic poisons or those affected by bacterial kidney disease. Mounib & Eisan (1969) observed that the differences in the activity of aminotransferases among fish and also between cells (eggs or sperm) and the suspending medium (egg fluid or seminal plasma) may be useful in tracing evolutionary differences in fish.

The present investigations report the distribution of aspartate and alanine aminotransferases and the scope of apparent transaminations in several fish and shellfish species.

Materials and Methods

Samples of different marine fish, shellfish and freshwater fish were obtained fresh for the analysis. The fish were beheaded, gutted and thoroughly cleaned with water. Unless otherwise specified, only white skeletal muscle was taken for analysis. At least five or more individual numbers of each species were analysed. The total aminotransferase activity was extracted by homogenizing the tissue with chilled 0.1 M phosphate buffer, pH 7.6 using a Torry-Brown homogenizer. The final proportion of tissue to buffer was 1:10. The homogenate was centrifuged at 10,000 g for 10 minutes in cold. The supernatant was

*Present address: Export Inspection Agency, Aman Chambers, 113, M. Karve Road, Bombay-400 004.

dialysed overnight in cold against frequent changes of 0.1 M phosphate buffer, pH 7.6 containing 10^{-3} g/l pyridoxal phosphate. It was noted that the loss in activity during dialysis could be prevented by incorporating pyridoxal phosphate in the dialysing medium. The dialysate, free from endogenous amino acids and keto acids was appropriately diluted for the assay of AAT and ALAT activities. AAT and ALAT activities were determined by the coupled enzyme reaction systems according to Bergmeyer & Bernt (1965) using Carl-Zeiss PMQ II spectrophotometer. The reaction mixture for the assay of AAT activity contained 2.3 ml of phosphate-aspartate solution (0.1 M phosphate buffer, pH 7.6, 2.5×10^{-1} M l-aspartate), 0.1 ml of 0.2 M 2-oxoglutarate, 0.5 ml of the tissue homogenate, 0.05 ml of 1.2×10^{-2} M NADH (reduced nicotinamide adenine dinucleotide-disodium salt) and 0.05 ml of malate dehydrogenase (0.5 mg protein/ml) previously dialysed against phosphate buffer to remove ammonium sulphate. For ALAT assay, the reaction mixture contained 1.8 ml of phosphate-alanine solution (0.1 M phosphate buffer pH 7.6, 0.1 M DL-alanine), 0.1 ml of 0.2 M 2-oxoglutarate, 1 ml of the tissue homogenate, 0.05 ml of NADH and 0.05 ml of lactate dehydrogenase (0.5 mg protein/ml) free from ammonium sulphate. One unit of enzyme activity is defined as

that activity which catalyses the oxidation of one micromole of substrate per minute at 30° C. The scope of transaminations to keto acids such as 2-oxoglutaric acid and pyruvic acid with various l-amino acids in the tissue homogenates of several fish species was investigated following the paper chromatographic method according to Rowsell (1962). The ascending development of the paper was performed using n-butanol/acetic acid/water (4:1:5) and n-propanol/water (4:1).

Results and Discussion

Table 1 reports the AAT and ALAT activities in the white skeletal muscle of several fish species. The AAT activity varied between 1.15 (Bombay duck) and 11.92 (horse mackerel). The ALAT activity varied from 0.35 (shark) to 2.26 (pomfret). The level of activities among individuals of the same species varied considerably. It is evident from the results that the level of AAT activity is comparatively higher than that of ALAT activity. It is seen that aminotransferase activity is relatively higher in some varieties such as horse mackerel, Indian mackerel, seer fish and cat fish. It is significant to note that enzyme activities, particularly AAT, are significantly greater in the red muscle of mackerels.

Table 1. Distribution of aspartate and alanine aminotransferases in the white skeletal muscle of different fishes

Species		U/g average ($\pm$ S. E.)	
		AAT	ALAT
<i>Megalaspis cordyla</i> (Horse mackerel)	i) White muscle	11.92 (2.3)	0.37 (0.07)
	ii) Red muscle	40.76 (5.5)	1.75 (0.5)
<i>Rastrelliger kanagurta</i> (Indian mackerel)	i) White muscle	8.89 (0.82)	0.95 (0.18)
	ii) Red muscle	49.25 (4.20)	2.52 (0.8)
<i>Scomberomorus guttatus</i> (Seer fish)		5.40 (1.4)	2.15 (0.7)
<i>Pampus argenteus</i> (Pomfret)		3.88 (0.68)	2.26 (0.74)
<i>Otolithus argenteus</i> (Croaker)		3.50 (1.65)	0.85 (0.62)
<i>Psettodes erumei</i> (Flat fish)		3.00 (1.1)	—
<i>Trichiurus savala</i> (Ribbon fish)		4.70 (1.8)	1.53 (1.3)
<i>Trachysurus dussumieri</i> (Cat fish)		5.50 (2.0)	1.30 (0.91)
<i>Harpodon nehereus</i> (Bombay duck)		1.15 (0.8)	0.49 (0.14)
<i>Scoliodon sorrakowah</i> (Shark)		3.59 (2.15)	0.35 (0.25)
<i>Cyprinus carpio</i> (Common carp)		2.98 (1.47)	—
<i>Penaeus monodon</i> (Prawn)		2.31 (1.17)	2.92 (1.82)

Hamm *et al.* (1969) noted that AAT and ALAT activities of bovine and porcine muscle were similar to the activities observed in the skeletal muscle of rat, rabbit and man. It is seen from the present studies that aminotransferase activity in fish muscle is relatively lower than the activity observed in other mammalian tissues.

The higher level of AAT activity seems to be associated with high myoglobin content of muscle (red muscle). Narawane (1967) obtained higher level of AAT and ALAT activities in lateral red muscles of two freshwater species of fish. Hamm (1969) observed a highly significant correlation between either AAT or ALAT activity and the amount of pigments in bovine and porcine tissues. Similarly, the red muscle of the carp showed higher activity than the white muscle (Masic & Hamm, 1971).

The extent of apparent transaminations in the skeletal muscle of seven species of fish and a crustacean using 16 different amino acids and 2-oxoglutaric acid as the amino group acceptor is presented in Table 2.

The results indicate that fish muscle catalyse transamination of several 1-amino acids such as leucine, valine, ornithine, tyrosine,

tryptophan, phenylalanine, methionine and lysine. In addition, some species show positive reaction utilising arginine, histidine and proline, though in traces. Transaminations involving cystine, glycine, serine, threonine and B-alanine could not be detected. Table 3 reports the occurrence of transaminations to pyruvic acid as the amino group acceptor. It is evident from the results that fish muscle catalyse the transaminations to pyruvic acid with very few amino acids. The possibility of coupling two different transamination reactions to indicate 'apparent' reaction (Guirad & Snell, 1964) is unlikely in the present studies since there were no chromatographically detectable amino acids or keto acids in the dialysed tissue homogenates. The study of Siebert *et al.* (1965) indicated that transaminations to 2-oxoglutaric acid were prominent in cod muscle as compared to pyruvic acid as the amino group acceptor. Creach (1967) detected transaminations to 2-oxoglutaric acid with leucine, isoleucine, valine, tyrosine, ornithine, phenylalanine and arginine in addition to aspartic acid and alanine in the tissues of carp. A survey of apparent transaminations in dialysed homogenates of brain, heart, kidney, liver and muscle of salmon indicated that AAT and ALAT were active in all tissues and

Table 2. Transaminations to 2-oxoglutaric acid in the skeletal muscle of different fishes and crab

Amino acids	Pomfret	Croaker	Flat fish	Horse mackerel	Shark	Rohu	Mrigal	Crab
Leucine	++	+++	++	++	++	++	++	++
Valine	++	++	++	++	++	++	++	++
Ornithine	+++	+++	+	++	+	+	+	++
Methionine	+	+	+	+	(t)	(t)	(t)	+
Tyrosine	+	+	(t)	+	(t)	(t)	(t)	+
Tryptophan	+	+	+	+	(t)	(t)	+	+
Phenylalanine	++	++	+	+	+	+	+	+
Lysine	(t)	(t)	(t)	(t)	(t)	(t)	(t)	(t)
Arginine	(t)	+	(t)	+	(t)	(t)	—	—
Histidine	(t)	(t)	+	(t)	(t)	—	—	—
B-alanine	—	—	—	—	—	—	—	(t)
Proline	(t)	(t)	—	—	—	—	—	—
Cystine	—	—	—	—	—	—	—	—
Glycine	—	—	—	—	—	—	—	—
Serine	—	—	—	—	—	—	—	—
Threonine	—	—	—	—	—	—	—	—

+ present, (t) traces, — absent

Table 3. *Transaminations to pyruvic acid in the skeletal muscle of different fishes and crab*

Amino acids	Pomfret	Croaker	Flatfish	Horse mackerel	Shark	Rohu	Mrigal	Crab
Leucine	+	+	—	—	+	—	—	+
Valine	+	+	+	—	+	—	—	+
Ornithine	+	+	+	—	—	—	—	—
Methionine	—	+	—	—	—	—	—	—
Tyrosine	—	+	—	+	—	—	—	—
Tryptophan	+	+	+	—	+	—	—	+
Phenyl alanine	+	+	+	+	+	—	—	+
Lysine	+	+	+	+	—	—	—	—
Arginine	—	—	—	—	—	—	—	—
Histidine	—	—	—	—	—	—	—	—
B-alanine	—	—	—	—	—	—	—	—
Proline	—	—	—	—	—	—	—	—
Cystine	—	—	—	—	—	—	—	—
Glycine	—	—	+	+	—	—	—	—
Serine	—	—	—	—	—	—	—	—
Threonine	—	—	—	—	—	—	—	—

+ present, — absent

that 2-oxoglutaric acid had wider scope as the amino group acceptor (Bell, 1968). This is in agreement with Cammarata & Cohen (1950) and Cohen & Sallach (1961) on other animal tissues.

The presence of several transamination mechanisms in fish probably account for the significant role played by these reactions in the biosynthesis or transformation of a number of amino acids and keto acids in fish *post-mortem* and in the metabolism of live fish. Siebert *et al.* (1965) observed that cod muscle contained active proteolytic enzymes capable of breaking proteins to yield free amino acids. It is probable that several interconversions of amino acids take place during storage and eventual spoilage of fish. Murray & Burt (1974) observed that transamination reactions were responsible for marked changes in the levels of aspartic acid, glutamic acid, alanine, valine, leucine and isoleucine in the extracts of cod muscle.

Chhatbar & Velankar (1977) noted that freezing and thawing of fish resulted in the release of mitochondrial isozyme of AAT, based on which an objective test to distinguish fresh, unfrozen fish from frozen and thawed fish was suggested. A critical study of various transamination reactions

in different fish species is probably of significance from the aspects of *post-mortem* handling and preservation of fish.

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The Triglyceride Fatty Acids from Heart Lipids of *Puntius Sarana* and Their Variation with Different Sizes

RITA MITRA and RAMJI D. DUA

Biochemistry Laboratory, Indian Institute of Technology, New Delhi-110 029

The triglyceride fatty acid components from the heart lipid of *Puntius sarana* of different sizes have been characterized by thin-layer and gas liquid chromatography. C₁₀ to C₂₄ acids including both odd-numbered and branched-chain acids were detected. The major constituents were ante-iso C₁₀, C₁₀, C_{12:2}, C₁₄, C₁₆, C_{16:1}, C₁₇, C₁₈, C_{18:1}, C_{18:2}, C_{18:3} and C_{20:4} while twenty other acids were detected in lower proportion. The composition of these acids and their variation with size of fish have been investigated and discussed.

The present work was undertaken as a part of the programme under progress in this laboratory to understand the triglyceride fatty acids and their variations with size of fish in various organs. *P. sarana* is an economically important fresh water food fish in India. This species has not been investigated for the fatty acid composition. Jafri & Qasim (1965) reported only the total liver fat content from *P. sarana* along with other carps.

Materials and Methods

All the solvents and the chemicals were analar grade (BDH/E. Merck). The solvents were freshly redistilled under nitrogen before use. The fatty acid methyl ester standards were procured from Sigma Chemicals Company (USA). The fish were collected from the same locale of river Jamuna at Delhi during a three week period in October. Only male fish were selected and transported in ice. Fish were sorted out into three different groups on the basis of their average length (10.2, 16.4, and 23.7 cm) and their hearts were dissected and stored at -20°C until used.

Extraction of lipids

The weighed heart tissue samples were extracted in a homogenizer using chloroform/methanol mixture (Bligh & Dyer, 1959). The lipid extract was dried over anhydrous sodium sulphate, filtered and stored at -20°C in a nitrogen flushed glass stoppered volumetric flask which was kept filled to the neck with solvent until analysed.

For storage longer than a week, an antioxidant BHT (2, 6 di-*t*-butyl 4 methyl-phenol) was added.

Separation of neutral lipids from phospholipids

The solvent from the total lipids was removed at 30°C under a mild vacuum in a Buchi rotary evaporator. The residue was taken up in 40-60°C petroleum ether and again concentrated to a small volume under nitrogen and the phospholipids were separated out by acetone precipitation (Kates, 1972) and neutral lipids were esterified after saponification by boron trifluoride method (Metcalf *et al.*, 1966).

Thin layer chromatography

Methyl esters were separated and identified by reversed phase TLC using 0.5 mm thick kieselguhr G plates impregnated with 10% v/v liquid paraffin in petroleum ether (60-80°C) at 5°C and developed in nitromethane acetonitrile acetic acid (15:10:10) (Hammonds & Shone, 1964) and by argentation TLC using 10% silver nitrate impregnated 0.5 mm thick silica gel G developed in (i) diethyl ether-hexane (15:85) (Mangold, 1969) and (ii) benzene (Linko & Karinkanta, 1970).

Gas liquid chromatography

Methyl esters were analysed on Perkin Elmer 900 model, dual column chromatograph, equipped with both automatic digital and disc integrator, using flame ionisation detector and employing either

polyester (polar) or silicone (non polar) column with the following working conditions. Polyester column: Stainless steel column (3.6 m x 2 mm i.d.) packed with chromosorb W (mesh 80-100) impregnated with 20% (w/w) diethylene glycol succinate (DEGS); column temperature 200°C; nitrogen flow rate 20 ml/minute; sample size 0.3 to 2 μ l (0.2 to 0.4% hexane solution). Silicone column: Stainless steel column (2.4 m x 2 mm i.d.) packed with gas chromo Q (90-100 mesh) impregnated with 5% (w/w) silicone rubber gum (SE 30) programmed from 180° to 320°C at the rate of 6°C per minute with initial and final hold time of 3 and 6 min respectively; nitrogen flow rate 20 ml/min; sample size 0.4 to 1.5 μ l.

Peak identification

Gas chromatograms of authentic samples were regularly run under exactly identical conditions in all cases. Sample peaks were identified by $\log_{10}$ relative retention time versus carbon number (James & Martin, 1956) and $\log_{10}$ relative retention temperature versus carbon number (Martin *et al.*, 1961) plotting procedures.

Unsaturated acids were confirmed by hydrogenating the methyl esters in methanol in the presence of platinum oxide catalyst and rechromatographing on the DEGS column under identical conditions. Absence of cyclopropane fatty acids and presence of branched-chain acids was established by rechromatographing the hydrogenated samples after bromination in diethyl ether (Kates, 1972).

Determination of percentage composition

The area counts were printed out by automatic digital integrator. Known standards were run regularly to check the linear response of the instrument. The percentage composition of each component was determined by area normalization as follows:

$$\text{Percentage of a component} = \frac{\text{area of that peak} \times 100}{\text{total area of all the component peaks}}$$

Results and Discussion

The number of fish used in each sample, the weight of the organs and the yield of the oil are given in Table 1. There was only limited variation in the lipid content of the heart (12.6%). There was slight fall in the 16.4 cm size (9.4%).

Table 1. Total lipids from the heart of *P. sarana* of different size groups

Average length cm	No. of fish	Total weight of heart g	% Lipid in heart w/w
10.2	103	5.3	11.3
16.4	112	36.9	9.4
23.7	49	33.3	12.6

The fatty acid methyl esters were analysed. As a few peaks in the chromatogram could not be identified unambiguously, the samples were hydrogenated and rechromatographed on DEGS column under identical conditions, confirming the presence of $C_{12:1}$, $C_{12:2}$, $C_{16:1}$, $C_{16:2}$, $C_{17:1}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ as their peaks shifted to the position normally occupied by the corresponding saturated fatty acids methyl esters. It was suspected from $\log_{10}$ plot that the peak for $C_{20:4}$ could be either $C_{22:1}$ or $C_{20:3}$ or $C_{20:4}$. Hydrogenation confirmed that it was not $C_{22:1}$. The possibility of $C_{20:3}$ was ruled out and this peak was identified as $C_{20:4}$ by following Ackman (1969). The identity of this acid was further confirmed by TLC.

A few peaks in the chromatograms did not shift their position after hydrogenation and therefore suspected to be either branched-chain acids or cyclopropane compounds. In the present investigation bromination of hydrogenated methyl esters did not lead to disappearance of any of the peaks on rechromatographing the sample and these peaks were identified as the following branched-chain fatty acids: Ante-iso $C_{10:0}$, ante-iso $C_{11:0}$, iso $C_{12:0}$, ante-iso $C_{15:0}$, ante-iso $C_{16:0}$, ante-iso $C_{17:0}$ and ante-iso $C_{22:0}$.

All the samples were also analysed on SE 30 column to check the data obtained in DEGS column and also to verify if any acid of chain length higher than C_{22} was

missed in DEGS analysis. The peaks were identified from the plot of $\log_{10}$ relative retention temperature *versus* carbon number. The SE_{30} data of heart lipids from all the three sizes revealed the presence of $C_{19:1}$, iso $C_{20:0}$, ante-iso $C_{20:0}$, $C_{20:0}$, $C_{20:1}$, iso $C_{21:0}$, ante-iso $C_{21:0}$, iso $C_{22:0}$, ante-iso $C_{22:0}$, iso $C_{23:0}$, ante-iso $C_{23:0}$, and iso $C_{24:0}$. These acids were present in traces and were missed in DEGS analysis.

The GLC data for fatty acids from heart of different sizes of fish are compiled in Table 2. While comparing these fatty

acids it was observed that ante-iso $C_{11:0}$, iso $C_{20:0}$, ante-iso $C_{20:0}$, iso $C_{21:0}$, and iso $C_{22:0}$ were present only in the heart of 16.4 and 23.7 cm size fish but, absent in the 10.2 cm size fish heart. The $C_{10:0}$, $C_{19:1}$ and iso $C_{23:0}$ were present in the 10.2 and 16.4 cm sizes. The $C_{11:0}$, $C_{14:1}$, $C_{20:0}$, iso $C_{20:1}$ and $C_{24:0}$ were detected only in 10.2 cm size fish heart. The presence of $C_{22:0}$ and ante-iso $C_{23:0}$ were detected only in 23.7 and 16.4 cm size fish heart respectively.

The variations in the total percentage composition of various fatty acids in heart

Table 2. Estimation of triglyceride fatty acids of heart from various sizes of *P. sarana*

		Size groups cm		
Fatty acids		10.2	16.4	23.7
Ante-iso	10:0	6.16	3.88	4.82
	10:0	8.14	4.81	—
Ante-iso	11:0	—	3.76	4.31
	11:0	2.48	—	—
iso	12:0	Trace	Trace	Trace
	12:0	0.15	0.19	0.03
	12:1	Trace	0.09	Trace
	12:2	1.31	1.94	1.42
	13:0	Trace	Trace	Trace
	14:0	1.20	2.51	1.66
	14:1	Trace	—	—
Ante-iso	15:0	Trace	0.07	Trace
	15:0	0.65	0.56	0.06
Ante-iso	16:0	0.41	0.28	0.07
	16:0	16.26	24.78	26.45
	16:1	2.73	2.32	0.45
	16:2	Trace	0.12	0.04
Ante-iso	17:0	0.65	0.75	0.29
	17:0	1.74	1.73	0.24
	17:1	0.15	0.21	0.02
	18:0	12.12	0.08	10.46
	18:1	34.63	31.07	39.23
	18:2	2.57	5.07	2.09
	18:3	5.23	3.59	0.94
	19:1	Trace	Trace	—
iso	20:0	—	Trace	Trace
Ante-iso	20:0	—	Trace	Trace
	20:0	Trace	—	—
	20:1	Trace	—	—
	20:4	3.41	3.76	1.17
iso	21:0	—	Trace	Trace
Ante-iso	21:0	Trace	Trace	Trace
iso	22:0	—	Trace	Trace
Ante-iso	22:0	Trace	0.42	0.07
	22:0	—	—	0.09
iso	23:0	Trace	Trace	—
Ante-iso	23:0	—	Trace	—
iso	24:0	Trace	—	—

Table 3. Comparison of the triglyceride fatty acids in heart of *P. sarana* of different sizes determined by GLC

Average length cm	Saturated				Unsaturated			
	Straight chain		Branched chain		Mono- enoic %	Dienoic %	Trienoic %	Tetra- enoic %
	Even numbered %	Odd numbered %	iso %	Ante- iso %				
10.2	37.87	4.87	Trace	7.22	37.51	3.88	5.23	3.41
16.4	40.37	2.39	Trace	9.16	33.69	7.13	3.59	3.76
23.7	38.69	0.30	Trace	9.56	39.70	3.55	0.94	1.17

of *P. sarana* of different sizes were compiled in Table 3. The saturated fatty acids in heart neutral lipids varied from 48.55 to 51.82%. The total percentage of saturated even-numbered acids was the highest in 16.4 cm size fish (40.37%) against 37.87 and 38.69% in 10.2 and 23.7 cm size fish respectively. The percentage of $C_{10:0}$ was quite high in heart lipids of 10.2 cm (8.41%) and 16.4 cm (4.81%) size fish, while this acid could not be detected in the heart of 23.7 cm fish.

The percentage of saturated straight chain odd-numbered acids was the highest (4.87%) in 10.2 cm size fish heart. This percentage decreased in heart lipid with size (2.29% in 16.4 cm size and 0.3% in 23.7 cm size fish). The presence of $C_{11:0}$ was significant (2.48%) in heart from 10.2 cm fish while it was absent in the heart from larger varieties of fish.

Among the branched-chain acids the iso acids were present in traces, while the percentage of anteiso acids was significant and was found to increase with size (7.22% in 10.2 cm, 9.16% in 16.4 cm and 9.56% in 23.7 cm size fish).

The presence of odd-numbered fatty acids was first reported by Farquhar *et al.* (1959) in menhaden (*Brevoortia tyrannus*) oil and later confirmed by others (Ito & Fukuzumi, 1962, 1963; Klenk, *et al.*, 1963; Roubal, 1963). Morice & Shorland (1956) first demonstrated the presence of branched chain fatty acids in shark liver oil. Ackman *et al.* (1963) provisionally identified saturated odd numbered straight chain fatty acids in seal oil and later confirmed them along with branched-chain acids

in marine mammals and fish (Ackman & Sipos, 1965).

Sen & Sehlenk (1964) who found that mullet (*Mugil cephalus*) oil contained more than 25% straight-chain odd-numbered saturated, monounsaturated and polyunsaturated fatty acids suggested that these acids arise as a result of slow catabolism of the ingested odd-numbered fatty acids from phytoplanktons. On the other hand, Ackman (1965) held the view that odd-numbered fatty acids arise out of dietary thetin, present in a number of algae (Challenger, 1959). On the death of the host algae, thetin on enzymatic degradation may give rise to acrylic acid or propionic acid, a precursor for odd-numbered fatty acids, as was demonstrated by Tove (1959) in mice feeding experiments.

P. sarana feeds mainly on phytoplankton (green algae) and zooplankton consisting of crustacea, rotifers and insects (Sinha, 1972). The branched-chain and odd-numbered acids detected in this fish may be dietary, as Schlenk *et al.* (1960) reported the presence of these fatty acids in a fresh water alga, *Chlorella pyrenoidosa* while Klenk & Eberhagen (1962) found them in both phyto and zooplanktons. Their occurrence may also be expected in other fresh water organisms. Such fatty acids can also be biosynthesized by the fish from branched chain skeleton arising from the oxidative decarboxylation of branched-chain amino acids like valine, leucine and isoleucine and odd-numbered straight-chain from propionic or acrylic acid, the degradation products of thetin. The percentage of the monoenoic acids in the heart neutral lipid was the highest (39.7%) in the 23.7 cm size fish and

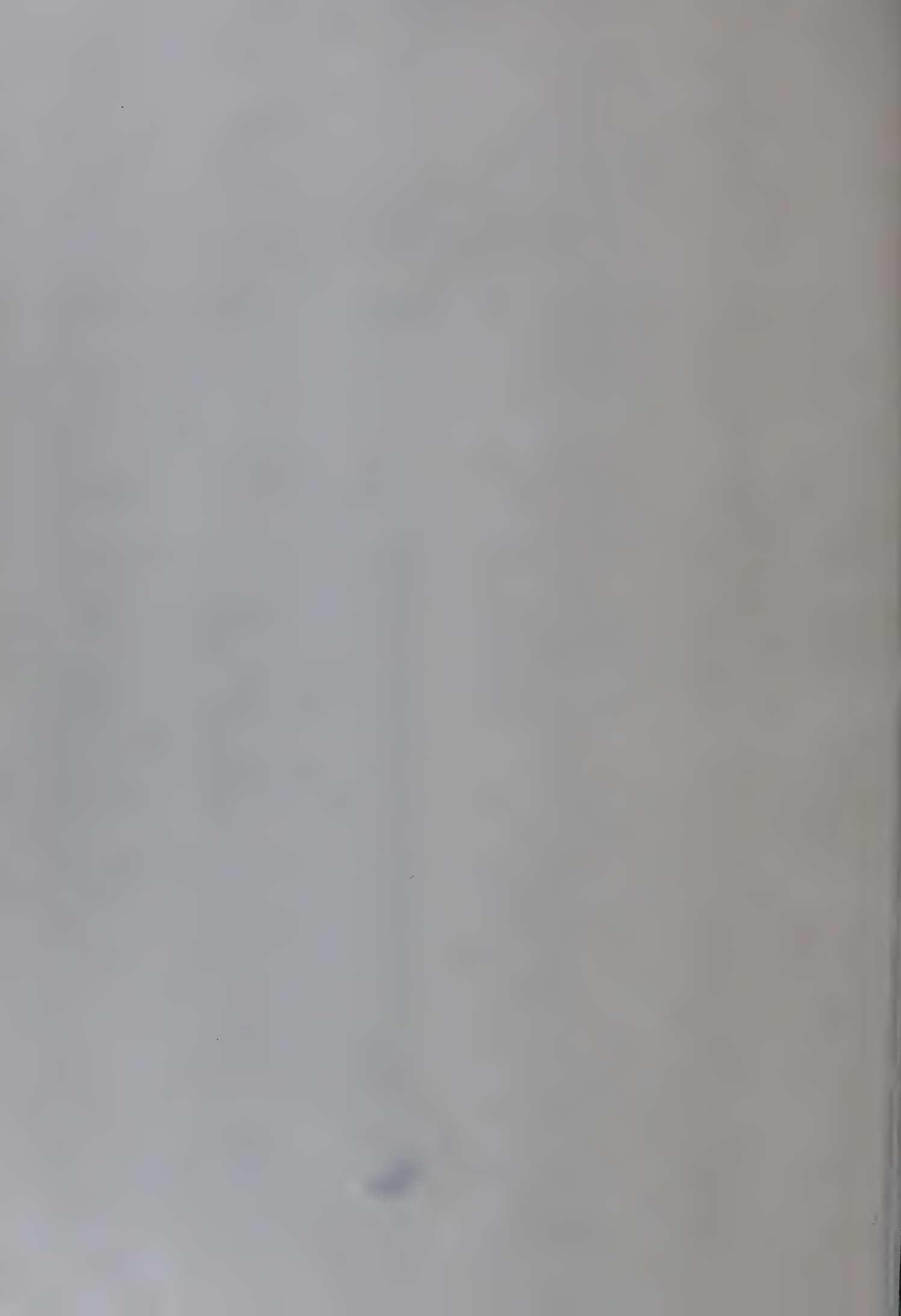
lowest (33.69%) in the 16.4 cm size fish. The major constituent was $C_{18:1}$ followed by $C_{16:1}$. The percentage of the dienoic acids in heart neutral lipid was the highest (7.13%) for 16.4 cm size fish while it was the lowest (3.55%) in the 23.7 cm fish. The major dienoic acid was $C_{18:2}$ followed by $C_{12:2}$ and $C_{16:2}$ in that order. The concentration of the trienoic acid ($C_{18:3}$) was the highest (5.23%) in the 10.2 cm fish heart and gradually decreased with size (from 3.59 to 0.94%). The only tetraenoic acid detected in fish heart lipid was $C_{20:4}$ in all the three sizes, the highest percentage being in 16.4 cm size fish heart.

Thus it was noticed that the level of triglyceride fatty acids in heart varied with fish size within narrow limits. A trend in quantitative relations has been established, but comparison is not possible as similar studies on this organ in other species have not been made.

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NOTES

Penetration of Sodium Chloride During Prolonged Salting of Fish

Curing by salting and drying has been the oldest, simplest, cheapest and most widely practised method of preservation of fish throughout the world. Not much research has been done to study the progressive absorption of salt by the fish muscle during prolonged salting. Changes occurring in moisture and sodium chloride contents in the muscle of mackerel in gutted and split open forms while salting for 48 h in the ratio 5:1 at room temperature have been reported by Sen *et al.* (1961) and those in the split open fish under the same conditions but with varying proportions of salt to fish from 1:4 to 1:12 by Lahiri *et al.* (1961). Govindan (1969) studied similar changes in oil sardine and mackerel during different stages of salting and drying.

The present work deals with absorption of salt and expulsion of moisture in threadfin bream (*Synagris japonicus*), jew fish (*Sciaenids sp.*) and lactarius (*Lactarius lactarius*) during prolonged salting. The fishes used in this study were procured fresh and handled immediately. They were gutted, gilled, cleaned well and salted as per details indicated. Moisture and sodium chloride were determined by the methods described in AOAC (1960).

Fig. 1 shows the changes in moisture and sodium chloride in the muscle of threadfin bream of average length 15 cm, gutted and salted in the ratios 1:3, 1:5 and 1:7 (salt to fish) at room temperature (28°C) for 4 days. Changes in moisture and sodium chloride in jew fish muscle (average length 20 cm), gutted and salted in the ratio 1:3 (salt to fish) for 5 days at 10°C, 28°C and 40°C are depicted in Fig. 2. Table 1 reports similar changes in gutted lactarius of average length 17 cm, salted in the ratio 1:3 at room temperature (38°C).

It is seen from Fig. 1 that the greater the proportion of salt employed, the quicker is its absorption and shedding of moisture

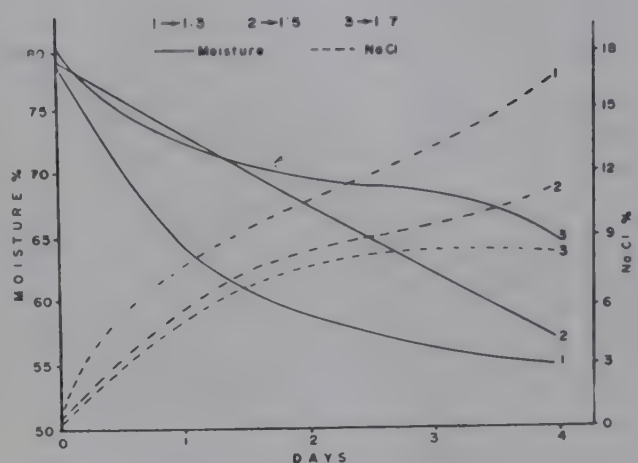


Fig. 1. Changes in moisture and sodium chloride in threadfin bream during salting at different salt ratios

by the muscle. The final levels of sodium chloride and moisture attained also are dependent on the salt ratio, moisture recording minimum and salt maximum values after 4 days with 1:3 ratio and *vice versa* with 1:7 ratio and a via media followed by 1:5 ratio. Temperature exerts a definite influence on rates of salt penetration and moisture loss as seen from Fig. 2. These are maximum at 40°C, minimum at 10°C

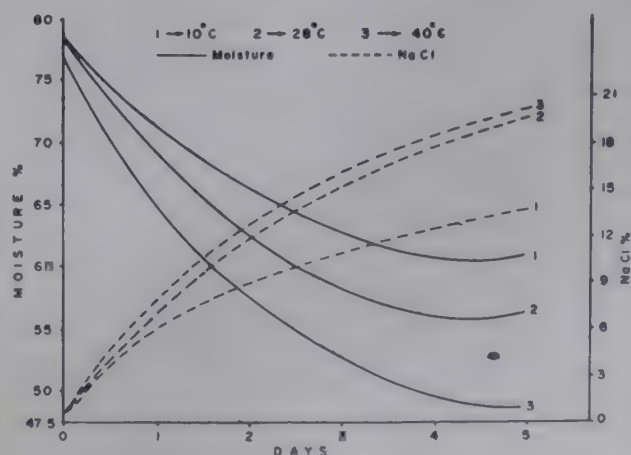


Fig. 2. Changes in moisture and sodium chloride in jew fish during salting at different temperatures

Table 1. *Changes in moisture and sodium chloride in Lactarius during salting for five days*

Salting days	Sodium chloride	Moisture
	%	%
Fresh	0.20	78.68
1	8.20	61.97
2	12.50	57.51
3	15.03	56.25
4	17.02	56.23
5	17.05	56.10

and in between at 28°C. In the case of Lactarius salted in the ratio 1:3, rates of penetration of salt and expulsion of moisture were rapid, even though the experiment was conducted at room temperature, which is attributable to the high ambient temperature of 38°C of the summer weather at Kakinada. Hence larger salt proportions and higher temperature of salting both accelerate the rates of absorption of salt and shedding of moisture by fish muscle during salt curing.

Kakinada Research Centre of Central Institute of Fisheries Technology, Kakinada-533 003

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D. NARAYANASWAMY*
C. V. NARASIMHA RAO**
T. K. GOVINDAN***

Present address: * Central Inland Fisheries Research Institute, Barrackpur, Calcutta-743 101

** Office of the Development Commissioner (Small Scale Industries), Ministry of Industry, Nirman Bhavan, New Delhi-110 011

*** Central Institute of Fisheries Technology, Cochin-682 029

Column Set Gill Net Fishing in Gobindsagar Reservoir

Znamensky (1967) observed the influence of temperature variations in movement of fishes. David *et al.* (1969) noticed better catches in bottom set nets during summer in Tungabhadra Reservoir. The present note reports a similar observation of the authors in Gobindsagar reservoir.

Kapron gill nets of identical design (Khan *et al.* 1975) were operated side by side as surface and column set under identical fishing conditions. The gill nets were column set at two metres below surface. Temperature of water of the fishing ground varied from 13 to 25°C during winter (November to January) and 20 to 33°C during summer (April to June).

winter, while it was 1.42 times more in surface set nets during summer. The catch consisted of the four main species of fishes viz. *L. diplostoma*, *L. bata*, *B. tor* and *M. seenghala*.

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Table 1. Variation in the catch of fishes during the winter and summer months

	Winter			Summer		
	No. of fish caught	Catch kg	Catch per 1000 sq. m net kg	No. of fish caught	Catch kg	Catch per 1000 sq. m net kg
Surface set gill net	71	55.20	10.21	169	206.65	33.15
Column set gill net	71	56.20	10.40	268	294.65	50.98

The catch per 1000 sq. m of column and surface set nets during winter and summer are presented in table 1.*

Table 1 shows no difference in catch for nets set at surface and column during

Znamensky, Yu. A (1967) *Report to the Government of India on the Development of Fishing Techniques in the Hirakud, Nisamsagar, Matatila and Gobindsagar Reservoirs and Their Affluents*. F. A. O. No. TA 2290, pp, 1-12

Burla Research Centre of Central Institute of Fisheries Technology, Burla-768 017

A. A. KHAN,
N. A. GEORGE*
O. P. PANDEY**

Present address: *Central Institute of Fisheries Technology, Cochin-682 029
**Indian Veterinary Research Institute, Izatnagar

Regd. No. 7720/64